

POUL FREUDENTHAL

EXPERIMENTAL RICKETS

INVESTIGATIONS ON THE GROWTH-PROMOTING,
FAT-SOLUBLE VITAMIN, SPECIALLY ELUCIDATED
BY ITS RELATION TO EXPERIMENTAL RICKETS.



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(L. S.)

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Denne Afhandling er af det lægevidenskabelige Fakultet antaget til offentlig at forsvares for den medicinske Doktorgrad.

København, d. 27. September 1927.

*A u g. W i m m e r,
h. a. dec.*

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PREFACE.

This investigation falls naturally into line with a series conducted in the course of time at the Hygienic Institute of the University of Copenhagen for the purpose of increasing and widening the doctrine of the stoichiometry and right composition of the food, in casu of the importance of vitamins for the growing organism.

While it was supposed when this work was commenced that the importance of the fat-soluble vitamin for the growth of young animal organisms, and for the prevention of xerophthalmia, was fairly elucidated, uncertainty and difference of opinion prevailed as to whether any vitamin is necessary for the prevention of rachitis, and in that case what rôle it plays. Hence, in these experiments I have mainly tried to throw light upon the relation between the fat-soluble vitamin and experimental rickets.

The work was carried out in the period between 1925 and 1926, when I was working as voluntary scientific assistant at the Hygienic Institute of the University. When I succeeded during this time in obtaining certain results and in completing this work, it is in great measure due to the director of the Institute, Professor L. S. Fridericia to whom I feel impelled to offer my most cordial thanks, partly for the good working conditions at the Institute, and partly for stimulating scientific impulses encouraging me in the work of each day and making it a pleasure. Further I am indebted to my Icelandic colleague and friend, Dr. Sk. V. Guðjónsson for much readiness to help and for many instructive discussions. My thanks are also due

to Miss Gudrun Johansen, whose helpfulness and clinical comprehension have been of great use to me.

Finally I beg Professor Dr. med. Johannes Fibiger to accept my most cordial thanks for the courtesy with which I was met at the Pathological Anatomical Institute of the University, and for the training I was enabled to obtain during my appointment as prosector.

Copenhagen, September 1927.

INTRODUCTION.

The aetiology of rickets has for years been the object of observations and investigations, theories and experiments, and as yet it has not been possible to prove the essential cause of the disease.

Every new observation which has necessitated new theories has at the same time made it possible to understand the results of earlier investigations and experiments. As the knowledge of the conditions from which they were derived, has been widened, it has become possible to draw more probable conclusions from the less imperfect premises.

Hence, with the knowledge which we now possess of the facts and conditions that are of importance for the results of the experiments and the right understanding of the investigations, we have the means of arriving at the indisputable results, and we understand that earlier investigators were obliged to draw conclusions with which it is now impossible to agree. I therefore think it warrantable to survey historically the leading theories that have prevailed in the course of time in the aetiology of rickets.

I.

HISTORICAL SURVEY.

Rickets, or the »English disease«, is the designation of an enormously widespread and frequent disease in children.

It is the consequences of the lingering continuance and progression of the disease, together with the deformations of the skeleton that may occur and get fixed during recovery, which have throughout been the conspicuous symptoms and have rendered it possible to give a special nosographic report already 300 years ago.

Rickets can be traced as far as mankind can be followed back. Thus we have bones deformed by rickets dating from the neolithic age. *H. Kjerrulf* (138) has described such discoveries of skeletons in Sweden, and *F. C. C. Hansen* (82) has shown rickets in bones originating from the first Northerners in Greenland. Many of the deformed and hunchbacked of early times, the often very intelligent jesters and dwarfs of the Middle Ages, are typical illustrations of rachitic skeleton deformations.

One of the first attempts to describe the disease was made by *I. B. Theodosius* in 1554, he describes a child aged seventeen months whose skin was pale, cool, and moist, and who could not walk yet, owing to weakness. Later on *Zacutis Lusitanus* mentions children who, because of softness of the bones, walked very late. *Ambroise Paré* describes deformations of the vertebral column and the rest of the skeleton, which we can now see are typical rickets.

The first extensive and more thorough description of the disease, however, originated in England. Here there had, in 1612

—1620, been observed numerous cases of rickets in the counties of Dorset and Sommerset, and later in the rest of England. Already at that time the English employed a method still in vogue in England. The Medical College of London appointed a special commission of eight members for the sole purpose of studying these new cases of disease and compiling a report on them.

The report was written by *Glisson* (70) and published in London in 1650. He gives the disease the name *Rachitis*, derived from the Greek word for spine, *ῥάχις*. He also uses the term »rickets« which is derived from the Norman word »riquet«, meaning hunchbacked or deformed. Soon it got the additional name »*morbus anglicus*« and was known and observed in the rest of the European countries.

A second and a third edition (71) were soon published (1660—1670), and here many particulars of the disease are thoroughly described and illustrated, especially the skeleton deformations. Already *Glisson* thinks that a damp, cold climate and poor nutrition produce the disease. But a great many extraneous symptoms and other infantile diseases enter into the descriptions, and only the investigations of later years have disengaged them.

In 1660 *Mayow* mentions softening of the bones.

The first attempt to distinguish between rickets in the initial stage and the consequences of the disease was made by *Levacher de Feutrie* in 1772, who sought the aetiology of the disease in deficient and wrong nutrition as well as bad hygiene.

The first description of the pathologico-anatomical picture was given by *Rufz de Lavison* (1834) who described the osteoid tissue, and by *I. Guérin* (1837—39) who tried to distinguish between the deformations of the initial stage and the softening of the bones from the later stage of eburneation. Many of the famous and well-known investigators of the past century have been occupied with these problems. Thus we may mention *Broca*, *Virchow*, *Ritter von Rittershain*, and *Kassowitz*, and this brings us down to our own day.

The Normal Bone Development and Normal Bone Growth.

Thus it was early understood that to explain the aetiology and pathogeny of rickets it was necessary to build on a thorough and exact knowledge of the normal development and normal growth of the bones as well as of the histological changes that occur during the development of the disease.

Further, the theories advanced must be based on this knowledge, and capable of explaining the pathologico-anatomical picture.

As is well known, ossification takes place in the cartilage bones, partly from the centre of the cartilage (enchondral ossification), partly from the surface of the cartilage, from the perichondrium (perichondral ossification).

The enchondral ossification begins with the growth of the hyaline cartilage cells, they become enlarged and divide in such a way that several cartilage cells appear in each cartilage capsule. The matrix between the cartilage cells becomes calcified by the deposition of an additional amount of lime salts. The cartilage cells thus enclosed are retracted from the capsule. In this way the ossific nucleus of the bone is formed.

The periosteum sends out proliferations towards the enchondral ossific nucleus, and it is observed that the calcified matrix is absorbed. Hereby is formed the primordial marrow cavity which, besides the periosteum cells and blood vessels grown into it, consists of the cartilage cells released by the decomposition of the matrix and the cartilage capsules. These cartilage cells take part in the forming of the future bone marrow, as well as in the ossification itself, being differentiated into big cells which often have proliferations, and which become embedded along the wall of the primordial marrow cavity as a single stratum.

Simultaneously with this process the perichondral ossification begins. The inner layer, which is rich in cells and highly vascular, deposits lime salts in the matrix and becomes osteogenetic tissue. When the cells differentiate into bone cells and the perichondrium into the periosteum, the perichondral bone is formed.

W. Dobbelt (35) assumes that a metaplasia of the cartilage cells never takes place in normal ossification, but that the ossification occurs entirely by cells, »osteoplasts«, derived from the marrow cavity.

The osteoblasts also occur on the inside of compacta, beneath periosteum.

The primordial marrow cavity becomes continually wider as the vessels growing forth together with the marrow cells continue the dissolution of the calcified matrix. In this way the ossification reaches the unchanged ends of the diaphysis.

Sooner or later an ossific nucleus will appear in the hyaline cartilage epiphysis.

At the ends of the diaphysis, the epiphyseal line, the zone with the resting hyaline cartilage cells, is seen most peripherally. Nearer the diaphysis the proliferous cartilage cell zone is found. Here there are two or three cartilage cells in each cartilage capsule. This is the »circular« cartilage proliferation zone, after this follows the cartilage proliferation with the cells arranged in the axial direction of the bone. And as now the cartilage cells get bigger and bigger, the column-like, regular structure in the axial direction gets more distinct.

These columns are surrounded by the matrix.

Still nearer the marrow cavity is found a rather narrow zone with big, vesicular cartilage capsules and retracted cartilage cells. Simultaneously the matrix here has resorbed lime salts, supposed to be absorbed as a calcium-albumin compound (*György*). This is the provisional calcification zone.

The provisional cartilage calcification zone covers only a small cell layer, as the blood vessels of the marrow cavity grow close up to these big vesicular cartilage capsules. These are dissolved, by which the cartilage cells become free. The matrix trabeculae which have separated the cartilage cell columns, still persist, though much contracted, as a system of calcified matrix trabeculae directed from the cartilage towards the marrow cavity, the ossification direction trabeculae. These are covered with a single layer of osteoblasts, which again are differentiated into bone cells, and simultaneously the matrix is transformed into osseous tissue (spongy bone trabeculae). These will still for some time enclose remnants of the original cartilaginous matrix column which do not ossify until later.

Since the investigations of *Kölliker* it is supposed that the osteoblasts secrete the ossifying matrix around them and thus get imbedded in it, while numerous proliferations from the osteoblasts connect with proliferations from other osteoblasts. In this way a system of communicating canals is built up in the ossifying matrix. In addition the osteoblasts differentiate the fibrils of the ossifying matrix, the lime-yielding fibrils. By the apposition of new bone layers the blood vessels present are thus enclosed in a system of osseous lamellae. In this way the Haversian canals are formed.

As is well known, the longitudinal growth takes place from the cartilaginous epiphysis. This cartilage ossifies definitively in the adult age. The growth of bone circumferentially takes place by the periosteal ossification process, which occurs in the inner layer of periosteum, the cambium layer, thick, crossing bundles of fibrils being differentiated, and deposition of lime salts taking place in the matrix, while simultaneously the periosteum cells are transformed into bone cells.

But during the growth of the bone and the gradual elaboration of its permanent form a resorptive action takes place. This occurs by means of big, often polynucleic cells, osteoclasts, which lie close to the bone trabeculae, and which, by resorption of the matrix, form Howship's lacunae.

The Pathologico-Anatomical Picture of Rickets.

Since the most conspicuous symptoms in rickets are the changes in the skeleton, and these nearly always appear at the age at which the growth of the children is most rapid, it will easily be understood that the object of earlier investigators was to describe and explain the histological skeleton deformations in the hope of finding the aetiologic factors.

According to descriptions by *Virchow* (258), *Kassowitz*, *Marfan*, *v. Recklinghausen* (200), *Carl Hochsinger* (110), *Schmorl* (218), *Spillmann* (236), and others, a hyperaemia, and apparently a proliferation of vessels wherever ossification takes place, is found in rickets. Thus *v. Recklinghausen*, *Schmorl*, and others, describe the penetration of the fine vessels, partly from the inner layer of the perichondrium, partly from the marrow cavity, into the cartilage proliferation zone (the »column zone«). Here no calcified matrix is present, as under normal circumstances, but a lime-free or but incompletely calcified matrix, in which a few calcified isles are still to be found, showing that it is the provisional calcification zone of the epiphysis (»Chondromalacia der Säulenzzone«, *v. Recklinghausen*).

The cartilage columns are not transformed into normal bone, but remain in greater or smaller columns or cartilage cell packets, without the capsule being dissolved, while the cartilage cells may grow more polygonal, like bone corpuscles, imbedded in the uncalcified homogeneous matrix. By this direct cartilage metaplasia is formed the »chondro-osteoid« or metaplastic osteoid described by *v. Recklinghausen*. *Schmorl* and *Dibbelt* (35) do not think that a direct cartilage metaplasia takes place.

But the osteoid tissue also arises when the cartilage capsules are dissolved, and the cells remain in the lime-free or lime-poor homogeneous matrix, which is thus unable to support the osteoblasts. This homogeneous lime-poor matrix, containing big polygonal cells, is as a rule described as osteoid tissue, and is found around the blood vessels and the marrow cavity. When thus the direction lines of the ossification remain uncalcified and are wasted by blood vessels and marrow cavities, it will be understood that instead of the regularly arranged structure of normal ossification an entirely deranged picture will be found in rickets. An irregular and disordered arrangement of the cartilage cells will be seen in the proliferation zone, a lack of calcification in the provisional proliferation zone, non-differentiated cartilage cell columns and isles, in places in the marrow cavity of the bone where, under normal circumstances, cartilage cells would never be found. Cartilage cells are present in an uncalcified matrix (chondro-osteoid). The marrow cavity is invested with bone-like but uncalcified lists and borders of homogeneous tissue containing oval or angular cells, i. e. osteoid tissue.

Pommer (193, 194), *Schmorl* (217), *I. P. McGowan* (176), and others, ascribe great importance to the contraction of muscles and tendons, weight and pressure, and other mechanical injuries to the bones and their proliferation zones, in producing the irregular course of the cartilage cell columns and the thickening of the epiphyseal lines.

Most of the investigators report that the new-formed uncalcified ossifying matrix (the osteoid tissue) is found to an increased extent. *Schmorl* reports that it is more plentifully formed in rapidly growing bones, and is likewise most plentiful in that part of the bone which grows quickest, but it is not an abnormally plentiful proliferation (cartilage growth), only a delayed demolition.

But in addition an increased resorption of previously established completely calcified bone tissue, partly of compacta, partly of spongy trabeculae, may be shown. These may either be transformed into osteoid tissue originating by halisteresis (i. e.

a decalcination process), or the bone tissue may be rarified, become osteoporotic. *Pommer* thinks that osteoporosis is developed by lacunar resorption of a greatly increased number of osteoclasts.

v. Recklinghausen, *Dibbelt*, *Vierordt*, and *Ribbert* attach much importance to the halisteresis, which *v. Recklinghausen* thinks he is able to prove by pointing out »Gitternfiguren« in the bone trabeculae. These are supposed to be developed by processes of demolition and emollition, thus onkosis (by which is understood a swelling of the bone corpuscles), which effects a »thrypsis« of the surrounding lamellae. This he has tried to show by special methods of staining.

Schmorl does not think that any real halisteresis takes place, while certain experiments on the metabolism in rickets and osteomalacia seem to have shown that a considerable calcium excretion may occur.

C. Funk, too, maintains that an intense resorption of already calcified bone does not belong to the picture of rickets, but on the contrary characterizes the experimental pseudo-rachitis.

When recovery commences, calcification of the osteoid tissue will begin in the places where it would have begun originally, if rickets had not been developed, thus not primarily at the limit between the broad epiphyseal line and the diaphysis, but in the disarranged cartilage cell columns and isles, so that a disorder of the enchondral ossification may be seen even long after healing has commenced and is apparently finished.

Thus only the second stage of rickets, the florid stage, is in question when a calcium-free ossifying matrix can be shown; if calcium has been deposited in the matrix, the disease has passed into the third stage, the healing stage.

It will thus easily be seen that distinct amounts of osteoid tissue can only be formed in growing organisms, for if growth has ceased, no new bone tissue will be formed, and thus no incompletely calcified bone tissue (osteoid tissue).

Hence it may be supposed that the osteoid tissue in rickets hardly arises from halisteresis, for if cessation of growth occurs in the rachitic organism, the bones become osteoporotic.

Furthermore, the osteoid tissue is found in a very moderate degree in rachitis tarda.

Hence a distinction has been drawn between two forms of rickets according to the amount of the osteoid tissue, viz. the hyperplastic form with great thickening of the epiphysis, observed in well-nourished, often fat, but pale children whose growth has been fairly good, and the osteoporotic form, with pronounced craniotabes, without any great enlargement of the epiphysis, but often with soft bones (costae) or very brittle bones, as met with in thin, pale, emaciated rickety children. These forms have especially been maintained by *Siebert*, *Schmorl*, and *Dibbelt*, who have shown typical Howship's lacunae and perforated canals in the osteoid matrix.

Stoeltzner claims that a distinction between the two forms is not justifiable, since he has observed that such cachectic children most frequently get pronounced rickets as soon as they thrive and begin to grow.

Nor does *Korenchevsky* (140) find any essential difference. He supposes that the cachectic children have rachitis, but in a latent form.

Erdheim remarks that the cessation of growth is an advantage to the rickety children, as a small skeleton is far more advantageous to them than a larger but soft skeleton. This theory has been practically utilized by *Jundell* (131), *Czerny*, *Monrad* and others.

Osteoporosis shows a rather intimate connection with clinical rachitis, but it is especially during the attempts hitherto made to produce rickets experimentally that its great influence on the histological picture has been discovered.

Osteoporosis consists in a rarefication of the bone, subperiosteally as well as in the trabeculae of spongiosa and around the Haversian canals. The bone tissue diminishes and disappears.

This process takes place partly by the increased resorption of an increased amount of osteoclasts (*G. Pommer* (193, 194)), partly by the absence of apposition, or by a combination of both processes.

Schmorl does not attach any particular importance to the osteoporotic processes, regarding osteoporosis in experimental rickets as one of the deviations from human rickets.

In new-born children *Friedleben* has often found deficient calcification of the cranium, and *Schwalbe* thinks that a physiological osteoporosis is found in children in the first months after birth.

Later *Schmorl* (217) maintained that in cases of prolonged and severe rickets with severe digestive disturbances, osteoporosis may be found, and *Stoeltzner*, *Dibbelt*, and *Kassowitz* are even of the opinion that rachitic bones are usually more or less osteoporotic and that osteoporosis is an important component in the picture of rickets and osteomalacia.

Schmorl and *Stoeltzner* consider that the cause of osteoporosis lies principally in the reduced formation of new bone, since cartilage proliferation formed during the rachitic conditions remains in this stage and does not, as under normal conditions, disappear and become replaced by osseous tissue. They think, however, that resorption of osteoid tissue only takes place to a certain extent.

Ziegler considers rickets to be a general disorder of nutrition, which occurs during childhood, and is characterized anatomically by increased bone resorption, deficient calcification of the cartilage, and the formation and persistence of imperfect and uncalcified bone or osteoid tissue.

And, as a matter of fact, a great number of the earlier experiments seem to show this connection.

Shipley, *Park*, *McCollum* & *Simmonds* (227) observe in experimental animals, kept on their special rickets-producing diet, that the growth of the bone has often ceased or been inhibited, that the bones are mostly osteoporotic when the animal has ceased to grow or has lost weight in the last half of the experiment. They maintain that, for the development of rickets, growth is necessary. If the conditions of the experiments are such that growth does not take place, rickets cannot be developed either, and so there is reason to expect that the rickets produced will disappear and osteoporosis develop.

They also showed that, if such animals with experimental rickets were deprived of food for from 3 to 5 days, fairly considerable deposits of calcium salts were found in the formerly calcium-free zone of proliferation. This the authors consider as due to increased oxidation caused by starvation, by which phosphorus is liberated, which is supposed to replace the deficiency of phosphorus in the diet, and thus bring about what they call the »healing of rickets by starvation«.

Korenchevsky (140) is of opinion that this phenomenon cannot be considered a healing of rickets, but merely as a change of rickets to »the latent form«.

Considering the pathogeny, *Pommer* and *Heubner* assume that it is the cartilage cells produced in an increased number which prevent and retard the dissolution of the capsules of the cartilage cells in the zone of provisional calcification, while *Schmorl* thinks there is no question of any increase in the number of cartilage cells formed, but that they remain in a less developed stage of differentiation. Owing to this, these cells are retained in the cartilage for an abnormally long time, while the formation of new cartilage cells continues. *Schmorl* and *Erdheim*, however, assume that the formation of new cartilage cells is likewise retarded. Even if it has been thought that a partial or complete lack of calcium is to be found in the zone of provisional calcification, it has been proved by *Wieland* (269), *Stoeltzner*, and others that a deposition of calcium under the periosteum takes place in rickets in children. *Erdheim* observed it in spontaneous rickets in rats, and by *Korenchevsky* (140) it was observed in experimental rickets.

Erdheim (52) supposes that some calcification and an enlargement of the zone of provisional calcification may occur in rickets, if only the dissolution produced by the blood vessels and marrow cavity of the zone of provisional calcification proceeds more slowly than the deposition of calcium in that zone.

Korenchevsky has observed in his experiments that when growth has ceased, either physiologically owing to advanced age or pathologically owing to an insufficient diet, a sparse formation of spongy trabeculae and simultaneously a more or

less normal saturation with lime of the zone of provisional calcification may occur. But he also mentions that the whole zone of proliferous cartilage was most frequently smaller than the normal, and the cartilage cells smaller too.

In rickets will be found, now a fairly intact continuation of the cells in the zone of cartilage proliferation, and now deep and disordered growth of the blood vessels of the marrow cavity into the cartilage. This *Schmorl* tries to explain as caused by deficient calcification of the provisional calcification zone, while *Korenchewsky* and others attach most importance to mechanical injuries.

Schmorl, *Pommer*, *Erdheim*, and *Korenchewsky* all maintain that there is no essential difference between rachitis, rachitis tarda, and osteomalacia. The differences are only due to growth, slow growth or arrested growth respectively.

The observations of *Siegert*, *Schmorl*, *Stoeltzner*, and *Erdheim*, that the rachitic changes are more pronounced in well-nourished children, less in cases of malnutrition, are in accordance herewith.

Also other phenomena have been pointed out in the histological picture. The French school, in particular, attaches much importance to the changes in most of the other organs in rachitis. Thus *Hutinel*, *Marfan*, *Spillmann* (236) mention certain general non-specific changes, viz. pale liver, slightly enlarged kidneys, dilated stomach and intestines, but in addition they describe striking changes in the marrow cavity of the bones.

Already *Guérin*, *Broca*, and *Virchow* have maintained that the bone marrow in rickets is poor in cells, and fibrous, and *McGowan* (176), too, describes fibrous marrow. This may also be found in advanced cases.

Pommer, *Schmorl*, and *Stoeltzner*, on the other hand, describe it as rich in cells, lymphoid, »hyperplastic«.

Ziegler distinguishes sharply between a cell-rich lymphoid part in the middle of the marrow cavity, and an osteoblast-containing fibrous part which can be found round the osseous trabeculae. This he calls the endost. *Pommer*, *Schmorl* and *v. Recklinghausen* do not attach much importance to the said endost and its relation to the osteoid tissue.

Pommer describes the bone marrow in rickets as »red marrow« with big blood vessels and numerous erythroblasts; he compares it with the foetal bone marrow and explains this phenomenon as a consequence of the numerous irritants which act on the abnormally soft skeleton. *Kassowitz* also draws attention to the hyperaemia of the bone marrow.

But it is especially *Marfan*, *Baudouin & Feuillé* (162), and *Hutinel & Tixier* (128) and *Jacques Debray* (34) who have described quantitative as well as qualitative changes of the bone marrow. Like *de Villa & Catia* (257) they find an increased erythroblastosis, an increased number of acidophilous cells and degeneration of neutrophilous myelocytes, a number of megakaryocytes, and small mononuclear lymphocytes. In addition they find hyperaemia. If the process continues further, fibrous marrow is formed. On the basis of this the authors argue that these alterations of the bones must rather be understood as conditioned by infection than by deficient calcification, for which reason they are inclined to think that infection or intoxication is the main aetiologic factor in rickets.

But *Kassowitz* (134, 135), too, has arrived at similar theories by explaining the deranged and considerable accumulation of cartilage cells with invasive growth of blood vessels as a consequence of specific rachitogenic irritation, perhaps infection, and *Hochsinger* (110) describes rickets as a chronic, irritative, universal bone disease.

The hypothesis that rickets is due to an infection or an intoxication is partly based on observations of »rachitic houses«, i. e. certain houses, in which for many years, in the various changing families, rickets has been found in nearly all the children. Further it has been observed that rickets may now and then occur to an extent and with a frequency, which makes one think of an endemic or even an epidemic, and finally of the observation of hyperplasia of the spleen in numerous cases of rickets. No characterizing histological investigations of such a hyperplasia are at hand.

But the hypothesis is especially based on the observation of various bacteria in the bone marrow of rachitic children. Thus

Mircoli has shown micro- and streptococci, *Spillmann*: bacillus pyogenes, b. coli, pneumococcus, pneumobacillus, but he made similar discoveries in the bone marrow in children with a normal skeleton. Neither did he succeed in developing rickets in experimental animals by the injection of these bacteria or their toxines.

The experiments of *Morpurgo* (184, 185) to which a certain importance was attached have now no interest for the question of the aetiology of rickets or osteomalacia, owing to his inattention to the question of diet.

But even *Paton*, after observing several cases of rickets in several members of the same family, is inclined to attach a great deal of importance to general infectious factors for the aetiology of rickets.

The experiments of *Korenchevsky* (140, 141) with various bacteria could not furnish support for such a hypothesis, not even in a case where spontaneous rickets occurred in a collection of rats.

The Hypotheses of the Aetiology of Rickets.

Having thus gone through the pathologico-anatomical investigations of rickets, we have at the same time seen some of the circumstances and conditions which the different investigators have supposed to be the aetiological factors in the development of rickets.

But before I go further into the investigations which have grown directly from the pathologico-anatomical basis, I shall mention such experiments which are mainly supported by clinical and anamnestic observations and studies.

It had been observed that rickets occurred most frequently in children at the ages of from 3 months to 2 years, with greater frequency in children artificially fed than in breast-fed children, and that it was more prevalent among the poorer classes than the rich, more frequent during the winter and spring months than in the summer and autumn months. Rickets is a disease

of the temperate zone seldom met with in the tropical or subtropical countries. And already *Glisson* attaches importance to a moist, cold climate for the occurrence of the disease.

v. *Hansemann* (81) maintains that rickets originates in children under the same conditions that cause its development in wild animals kept in zoological gardens. He considers it the result of confinement and the lack of fresh air and exercise. This he finds confirmed by the autopsies of children born in autumn and dying in the spring, who usually show definite or strongly marked signs of rickets. The severest form of rickets, however, is found in children born in the spring and dying about one year later, while in children born in spring and dying in the autumn signs of rickets are rarely discovered. This also agrees with the observations made by *Schmorl*.

Also the investigations of *Martha M. Eliot* (51) in urban districts of Newhaven chiefly inhabited by Negroes, Poles, Irishmen, and Italians, show that rickets is a seasonal disease, with its severest symptoms occurring in the winter and spring months. But she finds that the term of birth is without importance as to whether or not the child develops rickets. Rickets was found in 80—92 per cent. of the children examined.

These climatic, dietetic, and hygienic factors have, therefore, in the course of time been subjected to statistical investigations. Thus we may mention extensive investigations by *Corry Mann* (161), *Engel, L. Findlay & Miss Fergusson* (56), *Donald Paterson & Ruth Darby* (168), and *Rasmussen* (199).

The results of these investigations point in the direction of straitened circumstances, inadequate housing, inadequate nourishment, partly to the children, partly to the parents, especially the mother, or lack of exercise and fresh air, lack of sunlight, or previous infectious and contagious disease, or heredity, all factors which the various investigators think may in all probability be important as aetiologic factors in rickets.

Even though rickets is rare in the northern countries (Greenland (*Berthelsen*), Norway, Iceland,*) and rarely occurs

*) though according to Dr. *S. Guðjónsson* rickets is not rare in Iceland,

in the southern countries (Southern Italy, Greece, Spain, and the Tropics), but most frequently in the temperate zone, and there chiefly in the cities and centres of industry and most frequently in artificially fed children, rickets has also been observed in other countries, e. g. in Greenland, mostly in artificially fed children, and in India (*Hutchinson & Murphy* (126, 127)). The disease is also observed among well-to-do people, even in breast-fed children.

Already *Ritter von Rittershain* and *Pfeiffer* drew attention to the fact that rickets or a predisposition to rickets may be inherited. *Siegert* and *Heubner*, and *Kassowitz*, too, come to the same conclusion later on, as regards breast-fed children. More scientific investigation methods cannot attach any importance to heredity, but support the assumption that the health and nourishment of the mother have a certain influence on the development of rickets in her children.

Any real biological proof of heredity has never been given.

That race resistance does not play any rôle is shown by the fact that rickets is wide-spread among South Italians, Jews, and Negroes who are born in or have emigrated to the temperate zone.

However, investigations on this basis have proved inadequate to solve the problem of the aetiology in general. Conditions differ too much, there are too many factors, acting simultaneously, which partly produce and partly prevent rickets, so that it would be impossible to fix on the essential factor.

It may be supposed, therefore, that we shall come nearer to the solution of the question by studying the observations based on the conditions found in the rickety organism, and also such investigations and experiments which try the effect of one factor at a time.

The theories have primarily been based on the fact that rachitic bones have a lower ash content and higher water content than normal bones, and as calcium phosphate constitutes 86 per cent. of the bone-ash, it is again the deficient calcification which it has been attempted to explain.

I. Forster (58) showed the biological necessity of ash-com-

ponents in the nourishment, and found that the ash content of the organism increased with age, while *H. Brubacher* (21) found a considerably lower content of organic components in the bones of children with rickets than in normal bones.

This has led to extensive investigations of the calcium metabolism in normal as well as rachitic children, and of its importance as an aetiological factor.

From these investigations it appears that a great deal of the calcium administered is excreted with the faeces. Only a small part and under certain conditions sometimes a greater part, is excreted with the urine.

Harald H. Salvesen (207) has shown that calcium injected (intravenously) in children suffering from tetany will chiefly be excreted with the faeces, only 10 per cent. with the urine.

As calcium is a necessary component of the organism, it is necessary for the growing organism to retain a certain quantity of the calcium administered, and the more the quicker the growth is.

Korenchevsky (140) has collected what has appeared in the literature of analyses of milk from women with healthy children, 88 in all. 75 of these show a content of 0.03—0.049 per cent. of CaO. Two analyses show 0.08 per cent. and two others 0.029 per cent. Further there are analyses from 26 mothers with rachitic children and here the average is 0.0315 per cent. of CaO. Further it is mentioned by *Pfeiffer*, *Camener*, *Soeldner* and *Huncæus* that the ash-content of the milk is greater in the first month of lactation. *Pfeiffer* also showed that there was a diminished phosphorus content in the ash from »rachitic« mother milk (19.6 per cent. as against the normal 24.7 per cent.).

Now the investigations show that the percentage retention of calcium seems to be dependent on the form, and on the diet, in which the calcium occurs.

While thus the human milk is essentially poorer in calcium than the cow's milk, even taking into consideration the variously diluted milk with which the child is nourished according to its age, a considerably greater retention of calcium (60—70 per cent.) in children on a diet of woman's milk can be proved.

while the retention of calcium with a diet of cow's milk or a mixed artificial diet is found to be only 20.3—40.4 per cent.

Holt, Courtney & Fales (116) find that as the retention of calcium with cow's milk or artificial diet was 40.4—20.3 per cent., the minimum amount of CaO to be administered in order to reach balance is 0.09 gm CaO per diem per kilo.

In rickets the retention is considerably diminished, even in rachitic children fed with human milk a retention of only 15—31 per cent. instead of the normal 60 per cent. was found. Thus it is not surprising when it has been thought that the aetiology of rickets may be explained either by an insufficient supply of calcium or an insufficient assimilation or retention of calcium, or both together, perhaps in conjunction with an increased excretion of calcium.

Thus *Steinitz, Weigert, Schabad* and others have found that the calcium content may be reduced by one-third of the content at birth, in rachitic children. The daily decrease of calcium has been shown to reach considerable amounts, *Dibbelt* has found 0.339 gm CaO, *Orgler* 0.267 gm, and *Klotz* 0.41 gm CaO.

It was therefore expected that a negative calcium balance would be found in the rachitic organism, and it has often been found, too, in the very few experiments which have been made partly with artificially nourished partly with breast-fed children.

But it is far from all cases of rickets in which it has been possible to show a negative calcium balance.

Korenchevsky has carefully and critically gone through all the investigations hitherto made on the calcium metabolism, and he shows that out of 90 cases of normal children on a diet of cow's milk, in 79 cases a calcium supply greater than 0.09 gm CaO per kilo per diem was found, and in 11 cases less than 0.09 gm.

In 50 cases of rickets only 3 cases showed figures lower than or equal to 0.09 gm CaO per kilo per diem, while the supply in all the other cases was higher.

Thus in nearly all cases, normal as well as rachitic, it seems

that more than the physiological minimum demand has been supplied.

In breast-fed rachitic children a positive balance was only found in 2 out of 13 cases. And in rachitic children on cow's milk a positive calcium balance was only found in children between 7 and 24 months of age.

On a mixed artificial diet no distinct difference was found in the balance in rachitic and normal children.

In rickets in the stage of recovery a considerable retention of calcium was found, all these children were treated with cod-liver oil.

The slight positive balance, or in some cases even negative balance, which may be found in normal children, *Korenchevsky* thinks he can explain by supposing that rickets has already been present in the initial stage, (in a latent stage in several of the cases mentioned), in view of the fact that rachitis is very frequent at ages under one year.

That a positive balance has been shown in many of the cases, he explains by assuming that rachitis may be developed with a low positive balance if it is supposed that the deposition of calcium in the growing child is continued, but in a slighter degree than physiologically, so that the new-formed osseous tissue gets deficiently calcified. This is also in accordance with observations made by *L. Emmett Holt jr.*, *Victor la Mer* & *H. Bruce Chown* (117).

Furthermore *Korenchevsky* mentions that many of the metabolism experiments are of too short duration, and have therefore very likely been made during remissions in the development of rickets. Such remissions of the symptoms of the disease have been shown by *Schabad*, *Looser* and others.

But here, too, *Korenchevsky*, in accordance with *Schabad* attaches much weight to the fact that rachitis often commences as latent in a period when the child looks natural and healthy, and when perhaps the only pathological symptom to be found is in the calcium metabolism. As a result the clinical bone symptoms are developed. At this stage the calcium balance is perhaps

negative, whilst in the following stage, when the symptoms are pronounced, but the softness of the bones ceases, a slight positive balance will be found, which at last gets strongly positive when recovery commences with the deposition of calcium in the osteoid tissue, especially during cod-liver oil therapy.

That an insidious onset is likely also appears from the investigations of *Dibbelt* (36). He has proved a negative calcium balance in a child 21 days old, and in a child 35 days old, and *Grosser* (74) likewise in a child 4 weeks old, prematurely born.

As regards osteomalacia, a disease closely related to rickets, a negative calcium balance was found in 8 out of 13 cases collected by *Korenchevsky* from the literature. *Hotz* (118) points out that the positive balance in one case reported by *Neumann* (186) must be explained by commencing recovery.

In cases described by *Sauerbruch* (208), *Hotz* (118); and *His* a beneficial influence on the retention of calcium was effected by phosphorus and cod-liver oil therapy.

In one of the cases of *Neumann* (186) castration had a similar effect, but was without influence in the other two cases. *H. Fehling* (55) could not prove any difference in the Ca and P excretion before or after the operation. In most cases the phosphorus balance was positive. In 3 cases out of 4 recorded by *Lee Monroe Miles & Chih Tung-Feng* (179) a positive phosphorus balance was also found but a negative calcium balance. They showed that these balances vary with the seasons, and that A-vitamin can prevent and heal osteomalacia.

The Effect of the Introduction of Calcium into the Normal Organism.

When it has seemed possible from these investigations and considerations to draw the conclusion that the lack of calcium was the essential factor, it might also be supposed that an increased intake of calcium would influence the calcium metabolism favourably, perhaps even prevent the commencement of rickets or produce healing if rickets had already developed.

But *Neurath*, *Katzenellenbogen* and others did not find any increase of calcium in the blood after an increased intake in the case of children.

Voorhoeve (261, 262) gave large doses (2.7 gm CaO per diem) for several days to patients suffering from different diseases, and noticed that the calcium content of the blood increased, while he did not obtain this effect with smaller doses (0.55 gm per diem).

But for the rest all the investigators find only a temporary effect, thus *Heubner & Rona* (109), who determined the influence of calcium chloride introduced intravenously or subcutaneously or per rectum in cats, or by inhalation. On the other hand peroral intake seems to have a favourable effect, if the organism has for some time been kept on a calcium-poor diet.

The auto-experiment made by *R. Berg* (6) is of great importance in this connection since it shows the metabolism of calcium, phosphorus, and magnesium in humans.

But as *Korenchevsky* points out, the foundation of *Berg's* conclusions is shaken, since the diet was not only insufficient as regards Ca, but also, as we can now see, as regards fat-soluble vitamin. This circumstance may even be of great importance in an adult organism, the more so since the experiment covered a period of 92 days. During this period *Berg* lost 9.75 kilo in weight, though 2555 calories per diem were given, and he became neurasthenic and anaemic. *Berg* supposes that the symptoms may be explained by a poisoning of the organism by the excessive intake of HPO_4 and SO_4 . The calcium and phosphorus balance was negative. And while he did not see any favourable effect from the intake of calcium and phosphorus, calcium lactate had a certain improving influence on the calcium balance, but as soon as the intake of calcium ceased, the retention of calcium stopped.

On the other hand he found that lecithin had a favourable effect on the calcium balance.

The Effect of the Introduction of Calcium into the Rachitic Organism.

Schabad, Orgler (187), and others investigated the effects of a peroral intake of various calcium salts (-phosphate, -acetate, -citrate) on the calcium metabolism during rickets. These experiments showed distinctly that an intake of calcium during rickets is not able to improve a negative calcium balance, only in cases where rickets was in the recovery stage was it possible to show an increased retention of calcium. Hence they drew the conclusion that in the progressive stage of rickets the organism is unable to deposit calcium.

Schloss, too, (215) could not observe any improvement of a moderate positive calcium balance in a rickety child 2½ months old fed on human milk, in spite of the introduction of 1.2 gm of calcium acetate per os.

In the experiments of *Grosser* (74) the children were observed for several weeks (up to 9 weeks). He examined the influence of calcium acetate, calcium-glycero-phosphate, and chloride, and found only a rapidly decreasing »Scheinretention« of calcium. The glycero-phosphate introduced subcutaneously showed some effect, however, as the balance of calcium was kept positive for some time after the introduction.

Also *V. Korenchevsky & M. Carr* (144) were able, by experiments on rats on an A-vitamin-free diet, to show a small improvement of the calcification after injections of calcium-glycero-phosphate.

In some of his experiments *Dibbelt* (37) succeeded in improving the percentage content of CaO in »rickety mother milk« by the administration of calcium to the mother, while this could not be proved in the experiments of *Hunæus, Bardt & Edelstein*.

Moreover all experiments seem to show an essential difference between rickets and calcium starvation, the intake of calcium being ineffective in rickets, but effective in the latter disease.

Moreover, no decreased calcium content in the other tissue

of the organism, no general calcium paucity, has ever been shown in rickets (*Brubacher* (21), *Stoeltzner*, *Heubner*, *E. Voit* (259), *F. Voit*). But *Aron* maintains that variations in the calcium content of the other organs may be so small that they will fall within the limit of error, though he thinks that the lack of calcium may possibly be an aetiologic factor. For he maintains that the calcium in the bones plays a »passive« rôle, while in the rest of the tissue of the organism it plays an important physiological rôle and cannot be dispensed with, nor can it be withdrawn since it is bound to the organic albumin. Hence no reduction of the content of calcium in the muscles or the blood will be found.

The Effect of Cod-Liver Oil and Phosphorus on the Calcium Metabolism.

The causes of the altered calcium metabolism must therefore be sought in other factors than too small a supply of calcium, so *Schabad* (212, 213) made some rational examinations of the anti-rachitic remedy known from olden times. He investigated the effect of cod-liver oil on the calcium metabolism in rickets.

Schabad (212, 213) and *Schabad & Sorochowitsch* (214), *Schloss* (215), *Grosser* (74) examined the influence of the administration of calcium and cod-liver oil simultaneously.

In one of the cases of *Schloss* the separate administration of Ca-acetate and cod-liver oil seemed to be ineffective, while administration simultaneously increased the calcium retention.

Schabad also examined the influence of cod-liver oil with emulsion of phosphorus, and is inclined to attribute to the phosphorus the favourable effect on the calcium balance. Later he showed by his investigations that the principal factor is cod-liver oil, as the administration of phosphorus alone did not show any appreciable influence on the calcium metabolism. While all experiments showed that cod-liver oil rapidly and reliably influenced the calcium balance in a rickety organism, it was

without any perceptible influence in healthy children, a fact which has also been confirmed by *Schloss & Franck* (216). *Schabad* observes that in a rickety child without treatment the CaO retention was 4.79 per cent. of the CaO amount administered; by the addition of phosphorus, 10.11 per cent., of 10 gm sesame oil, 0.97 per cent., but by treatment with 10 gm cod-liver oil, 43.35 per cent., and by cod-liver oil to which was added phosphorus, 72.49 per cent. *Schabad* also observed that cod-liver oil improved the nitrogen, fat, and phosphate retention in a rickety organism.

Holt, Courtney & Fales (116) showed some advantageous effects of cod-liver oil.

Howland & Cramer (120), *Frans Faber* (53), and others made numerous investigations on the calcium and phosphorus content in the blood before and after treatment with cod-liver oil, and in florid rachitis most frequently found a normal calcium content, but a reduced phosphorus content. *Howland & Cramer* found that the phosphorus content of the blood increases greatly after treatment with cod-liver oil, while the calcium content is unchanged. Only when the calcium content of the blood was reduced beforehand, as most frequently found in tetany, did cod-liver oil cause an increase of the calcium content.

In the investigations of *Sauerbruch, Hotz, His* and others on osteomalacia, cod-liver oil produced the same favourable effect on the calcium metabolism.

The Influence of Various Diets on the Calcium Metabolism.

Cod-liver oil had thus proved able to heal rickets or at least improve the calcium metabolism, without its action being understood, so it was thought that the origin of rickets must be sought in an inadequate or disproportionate composition of the diet.

W. Cronheim & Erich Müller (30) and *Schabad* (211) had shown that an increased fat content in the diet caused an in-

creased loss of calcium in children. It was thought that great amounts of unresorbable soaps were formed in this way, but it could not be explained why cod-liver oil had not the same effect.

Other investigators (*Rothberg* (206)) had observed that an increase of carbohydrates in the food would likewise cause a diminished assimilation of calcium, though to a less extent than with a diet rich in fat. *Kochmann* and *Petzsch* (139) maintained that over-feeding, with fats as well as with carbohydrates and albumin, could disturb the calcium equilibrium by increasing the amounts of poisonous metabolic products. A large addition of calcium was therefore supposed to be required to re-establish the balance.

Freund (62) observed that malt-extract improved the retention of calcium, and *Mellanby* confirmed the efficiency of the same for puppies with experimental rickets.

But as the occurrence of rickets amongst children fed on cow's milk can hardly be the result of a real disproportion between the contents of fat, carbohydrates, and proteins in the diet, and much less so if the child is fed on woman's milk, attention was again directed towards the supplied and resorbed calcium amount as compared with the required amount.

According to the investigations of *Aron* (2), *Soeldner*, *Schaabad* (209), and *Korenchevsky*, for each gain of 100 gm of the organism of a breast-fed child, there will be deposited 1.0—1.25 gm CaO. If the CaO percentage in woman's milk is estimated at 0.043, and it is supposed that 70 per cent. is retained, it will be seen that the calcium demand of the child is greatest during the time when growth is quickest, i. e. in the first years of life, and especially the first 3—4—6 months of life. The calcium requirement even seems to be so great that on this estimate it hardly seems possible that it can be covered by the calcium supply in the first two months of life.

From the age of 3 months the balance seems to be established, but if the CaO percentage of the milk is lower than 0.043 and the retention less than 70 per cent., which were the most favourable figures, it will be seen that there may be a possibility of calcium starvation.

If cow's milk contains 0.158 per cent of CaO , and a child one month old is fed with one milk plus one water aa 400 c.c. per diem there will be 0.632 gm CaO in it. The retention for cow's milk is 30 per cent., hence 0.19 gm CaO will be retained daily, whilst the demand of calcium at this age is 0.30—0.25 gm; this can only be attained by a retention of 48—40 per cent.

If 3 milk plus 2 water, altogether 900 c.c. are given daily, the nourishment will contain 0.829 gm CaO , and by the retention of 30 per cent. the figure is only 0.248 gm.

This is also in accordance with the theory of *Aron* that over-fed children, i. e. children with excessive growth, in spite of a possibly normal calcium content of the milk, may suffer from calcium starvation, and with what *Schabad* points out, viz. that the percentage calcium content of the bones of a child of 5—6 months of age is less than in the bones of a new-born child. *Friedleben* observed a physiological bone resorption in the occiput in children of this age.

There is thus great probability that the child, in the first months of life is exposed, if not to an absolutely subminimal supply of calcium in the diet, still considering its known possibilities of resorption, to an insufficient deposition of calcium in the organism. This also seems apparent from the numerous clinical observations, partly of craniotabes, partly of congenital rickets in children. Thus congenital or early developed craniotabes (age, 2—3 months) has been shown. It is considered by most authors as the first stage in the pathogenesis of rickets, which need not, however, be followed by rickets, and need not even be caused by rickets (*Jules Comby* (27), *Barenberg & Bloomberg* (4), *Wilson & Seldowitz* (270)). *I. Axel Højer* (129) says that the deficient calcification must only be regarded as a quite ordinary delay of ossification.

It is possible, therefore, that calcium starvation, even if it cannot be considered an essential aetiologic factor may play a certain rôle in the pathogeny of rickets.

Ever since *Chossat* (26) made the first experiments by feeding pigeons with a diet poor in calcium, and produced bone fragility and osteoporosis, numerous experiments of the same sort have been made with dogs, guinea-pigs, lambs, and mon-

keys. Here I shall only mention experiments made by *Weiske & Wildt* (268), *Roloff*, and *E. Voit* (259).

The description of the histological changes of the skeleton may be somewhat like rickets, it is true, but the chief symptom seems to be osteoporosis. The first more thorough and critical histological investigation of such experiments was made by *Miwa & Stoeltzner* (180, 181). They call the picture pseudo-rachitic osteoporosis. This was confirmed by the experiments of *Aron & Sebaue*r (23). Here *Goetting* (79) made the histological examination and found the most essential feature of the picture to be a predominant calcium resorption as found in osteoporosis, while for rickets a deficient deposition of calcium is required.

In the experiments of *Dibbelt* (36, 38, 39) on puppies, the diet consisted of meat, fat, carbohydrates, and NaCl, the control animals received calcium phosphate in addition. The histological changes were various, were most like rickets in the animals fed on meat and carbohydrates, less in the animals fed on meat and fat. *Dibbelt* attached a certain importance for the development of rickets to the carbohydrates. *Schmorl* examined the changes of the bones and describes them as pseudo-rachitis, with pronounced osteoporosis, whilst he regards the presence of osteoid tissue as accidental.

The most rational experiments on the influence of a diet poor in calcium have been made by *Korenchevsky* (140) with a diet which, for young rats, is adequate as regards proteins as well as fat, carbohydrates, vitamins A and B, and as regards saline content. In this diet the calcium was left out. In this way *Korenchevsky* found that the skeleton had an increased water content and a reduced calcium content. The latter depended chiefly on the reduced deposition of calcium in the newly formed osseous tissue and in a less degree on the decreased amount of osseous tissue.

The weight tables show that these animals had a weight much below that of normal animals of the same age, also their skeletons were considerably smaller. Histologically he showed either osteoporosis or a picture similar to slight rachitis. The greatest changes he found in young animals (less than 35 days

old at the commencement of the experiment), the smallest if the animals had been put on diet two months old. The severest changes were found in animals whose mothers had been kept on a normal, but calcium poor diet during lactation.

To ascertain the difference between pseudo-rachitis and rachitis, several experiments on the influence of strontium on the metabolism have been made, it being supposed that strontium was to a certain degree able to replace calcium. *Helene Stoeltzner* (251) found that animals on a diet poor in calcium got osteoporosis, with only little osteoid tissue and a fairly good deposition of calcium in the newly formed osseous tissue, while animals on a diet poor in calcium but with an addition of SrPO_4 showed delayed bone resorption and considerable amounts of osteoid tissue. If these animals were given calcium per os, a rapid calcium deposition appeared in the osteoid tissue.

As *Stoeltzner* thinks that the osteoid tissue in genuine rickets is unable to absorb calcium, she explained the changes in animals fed on strontium as pseudo-rachitic.

Of the same opinion are *Voit* (259), *Aron & Sebauer* (3), and *Weiske* (267), they have examined the chemical composition of ash, Ca- and P-content in animals on a diet poor in calcium, the lowest figures were found for animals fed on strontium. *Weiske* therefore supposes that strontium causes a decalcination, perhaps halisteresis of the bones. The quantity of the organic bone matrix was found to have increased in such animals.

Voit and *Forster* found the calcium content of the muscles reduced, but this could not be proved by the experiments of *Weiske* and *Aron* and *Sebauer*.

Experiments on the Importance of Vitamins in the Metabolism during Rickets.

Hopkins (113) was the first to advance the conjecture (1906) that rickets is caused by a nutritive error which must be supposed to consist in the lack of a special organic factor in the diet. This was supported by the otherwise incomprehensible fact

that treatment with cod-liver oil produced a specific amelioration of the calcium balance in rickets.

But *Mellanby* was the first who tried to prove by experiments on puppies the existence of such an accessory food factor, which he called »the antirachitic vitamin«.

In the experiments of *Mellanby* from 1918—20 and 1921 he shows that the antirachitic factor is most likely identical with A-vitamin (the growth-promoting vitamin).

He tries to demonstrate this by showing that the quantitative fluctuations of both factors in various articles of food are identical and simultaneous, and that influences weakening or destroying the content of A-vitamin in the diet also have a weakening effect on the antirachitic qualities.

The diet on which *Mellanby* produces rickets in puppies consists of white bread ad libitum, separated milk (175—250 c.c.), yeast 5—10 gm, orange juice 5 c.c., linseed oil 10 c.c., sodium chloride 1—2 gm.

This diet contains sufficient B-vitamin (anti-neuritic) and C-vitamin (anti-scorbutic), but only a small amount of A-vitamin (growth-promoting), and, it must be supposed, too little calcium, especially for as rapidly growing animals as puppies.

According to the calculations of *Korenchevsky* the demand of calcium will only be covered by a retention of 70 per cent.

However, the deficit of calcium does not seem to have been the only decisive factor for the development of rickets, since 6 weeks old puppies, on the same diet and an addition of 5 gm $\text{Ca}_3(\text{PO}_4)_2$, got pronounced rickets with reduced calcium content in the bones, reduced from the normal 13 per cent. to 9.3 per cent of CaO.

On the other hand *Mellanby* observed that the addition of food-stuffs rich in the »antirachitic vitamin« prevents the incidence of rickets, and these food-stuffs were also able to heal experimental rickets. In a series of experiments he tries to determine which of the food-stuffs are especially rich in »antirachitic vitamin«.

In the succeeding years numerous reports appeared showing

the influence of the absence of A-vitamin on the incidence of rickets.

Thus we may mention the experiments of *Harden & Zilva* (83) on monkeys, *Tozer* (256) on guinea-pigs, *Mackay* (159) on chickens. Further numerous experiments on rats performed by *P. Shipley*, *E. Parks*, *E. C. McCollum & N. Simmonds* (227), *A. F. Hess*, *G. McCann & A. Pappenheimer* (91).

Golding, *Drummond* and *Coward*, and *S. S. Zilva* have investigated pigs.

V. Korenchewsky (140) in his experiments with an A-vitamin-free diet found that in young rats loss of weight, emaciation, and xerophthalmia appeared, though not constantly.

Some of the animals were killed, while still growing. At this stage emaciation or xerophthalmia were not found, 4 even gave birth to young ones. These animals showed pronounced macroscopical osteoporosis, while in a few, viz. 3 out of 41, he found severe rickets, and in 8 fractures of costae with callus. Microscopically, osteoporosis was found in 16 cases, slight rickets in 13, moderate in 11, and severe in one case.

However, he shows that the feeding of the young rats, and the feeding of the mother before the weaning, with a diet rich in A-vitamin, makes the young rats more capable of resistance to the unfavourable effects of an A-vitamin-free diet after the cessation of lactation. Further he shows that the diet of the mother during pregnancy plays an important rôle for the composition of the milk. He says that, if the nourishment has been rich in A-vitamin, P, and Ca during pregnancy and lactation, the young rats will be protected against the rachitis-producing effects of an A-vitamin-free diet after ablactation.

The chemical composition of the bones, however, shows that the preventive influence is not absolute. The animals seem to have got osteoporosis.

Furthermore *Korenchewsky* makes some investigations on the influence of the simultaneous absence of A-vitamin and calcium in an otherwise adequate diet. He finds that growth is retarded thereby, or stopped, and that the animals lose weight. The water content of the bone has increased much, and the calcium content is much reduced.

He claims that calcium starvation intensifies the rachitic bone changes, and that an A-vitamin-free and calcium-poor diet is the best to produce rachitic changes, distinctly and frequently, in the experimental animals.

From all these experiments the conclusion may be drawn that rachitic changes of the skeleton cannot be produced on a diet which is only deficient in A-vitamin, since in most of the cases osteoporosis was observed.

The experiments are characterized by the animals losing weight, getting emaciated, and losing their appetite, while *Mellanby's* puppies grew and did not get cachectic. The conditions of these experiments seem to get nearer to the conditions for the development of rickets in humans.

But these experiments contributed to shake the theories of the »antirachitic vitamin«, so much the more since *Findlay, Paton & Watson* (59) in 1918—1921, on the basis of their own experiments maintained that butter hardly contains any substance which prevents the development of rickets, since they see that rickets develops in puppies getting up to 14.5 gm of butter daily, while puppies that had a little milk-fat, but were kept in the country and often got exercise in fresh air escaped rachitis. Therefore they attributed most importance to deficient exercise and lack of fresh air as rachitogenic factors.

Later *Paton & Watson* (190) ascribe essential importance to cleanliness.

Mellanby shows that such puppies do not get rickets if the diet is good, while dogs kept in freedom and with plenty of exercise do get rickets when put on a rachitogenic diet.

The explanation why milk-fat or butter have in some cases proved able to prevent or heal rickets, while in other cases they have not had this effect, may be sought in numerous other experiments.

Thus it has been shown that A-vitamin in butter is easily destroyed by oxidation (*Hopkins* (114) and *Drummond & Coward* (43)).

Partly it has been shown by numerous experiments that the content of A-vitamin, determined by growth experiments on

rats, is absolutely dependent on the feeding of the cow. Furthermore that the content of A-vitamin in the milk is greatly dependent on the time of the year. This has been shown by *Drummond, Coward & Watson* (45), who were even able to prove that certain butter samples did not produce essentially better growth of the young experimental animals than such refined vegetable oils which are, as a rule, regarded as very poor in A-vitamin.

Chick, Dalyell, Hume, Mackay & Smith (25) found similar conditions in the milk of stall-fed cows, when they undertook their famous investigations on rickets in Vienna. They also showed that by treating 27 children with cod-liver oil the incidence of rickets could be prevented during the winter and spring months, while 14 out of 24 children, kept simultaneously under exactly the same conditions and on the same diet, but without cod-liver oil, got rickets.

Zilva & Miura (274) even found that butter may contain 250 times less A-vitamin than cod-liver oil, also determined by growth experiments on rats.

This greatly varying content of A-vitamin in ordinary and important food-stuffs, such as butter and milk, may explain much in the contradictory results drawn from the different experiments.

But from these experiments it appears, too, that if food-stuffs with a great content of A-vitamin are added to an insufficient diet in the experiments with rats, a deposition of calcium in the bones will soon be observed. Thus *Shipley, Park, McCollum, Simmonds & Parsons* (231) have observed this 3—8 days after giving an addition of 1—2 per cent of cod-liver oil to the rachitogenic diet, but cannot obtain similar results even with great additions of butter. They maintain that A-vitamin and the »antirachitic vitamin« are not identical. Further they maintain that cod-liver oil is rich in both these factors, while, on the contrary, butter only possesses the first. This is denied by *Mellanby*, who has always supposed that the two effective qualities were bound to the same food-stuff.

Shipley, Park, McCollum & Simmonds (227, 228, 229) used a diet deficient both in calcium and A-vitamin, and even if they

found that a 10—20 per cent. addition of butter did not have as good an effect as 1 per cent of cod-liver oil, all the same their animals did not prove normal as regards the consumption of food, fertility, and the ability to rear young, but if the content of calcium was increased, they showed that 3 per cent of butter was sufficient to produce a normal development.

On another diet deficient in A-vitamin and phosphorus (0.2 per cent P_2O_5) severe rickets was produced and could not be prevented by an addition of 10 per cent of butter, while 2 per cent of cod-liver oil was efficient. Therefore *McCollum* and his collaborators maintain that there must be two distinct organic factors operating in such fats, viz. A-vitamin, indispensable for growth and for the prevention of the development of keratomalacia and, secondly, the antirachitic factor, necessary for the normal development of the skeleton. The latter is found sparsely in butter, but in great amounts in cod-liver oil.

The observations on xerophthalmia are further used as an argument in favour of the two separate factors. This disease was described by *Mori* (183) in 1904 and later by the American investigators of vitamin, observed in rats kept on a diet poor in A-vitamin. But not until *C. E. Bloch* (14, 15) had thoroughly examined and described the disease, was xerophthalmia in children understood as a consequence of deficient food. *Bloch* supposed that the diet was deficient in certain factors occurring in several animal fats, and he also showed that xerophthalmia might be cured by the administration of cod-liver oil. *Svenn Monrad* (182) showed that a nosocomial xerosis conjunctivae might be cured by giving the children 10 gm of raw whole milk before each meal. For the sake of the experiment the children were kept for some time on the diet which had proved insufficient.

As several of the children with xerophthalmia were much emaciated and had been nourished for some time on a fat-poor diet and still did not show any clinical signs of rickets, *Bloch* and others with him supposed this to be a proof that rickets cannot be due to a lack of A-vitamin in the diet.

Another fact that may shake the theories of the »antirachitic vitamin« is the importance attributed to the content of phosphorus in the diet. It was founded on the observation that in acute and chronic poisoning with phosphorus an osteosclerosis may appear; this has also been shown by *Wegner* (226) in experiments on animals. *Kassowitz* (136) also thinks this has been proved, but he maintains that, if the diet is poor in calcium, the increased formation of new bone will manifest itself as rickets. Therefore he thinks it necessary to keep the supply of calcium salts at maximum, if phosphorus is to be used therapeutically. But his experiments further show that phosphorus possesses no advantage over cod-liver oil. Most of the earlier experiments by *Weiske* and *Wildt* and others cannot throw any light on the question as to whether phosphorus possesses any antirachitic effect, as the diets used have been deficient in salts as well as A-vitamin.

Shipley, *Park*, *McCollum* & *Simmonds* (228, 229), and other American investigators have adopted the lack of phosphorus as an essential factor in experimental rachitogenic diets. The diet of *Shipley* and *McCollum* consisted of rolled oats, linseed meal, dextrine or wheat, maize, gelatine, and wheat gluten, and sufficient amounts of CaCO_3 (0.67 gm or even more per 100 gm of food mixture). By this diet administered to young rats the authors in a number of animals obtained a picture which they describe as typical severe rickets, in other animals they only found osteoporosis, while they maintain that the calcification of the cartilage and bones was satisfactory. Some of the animals showed rickets complicated by osteoporosis.

From this and subsequent experiments they draw the conclusion that a diet rich in calcium does not prevent the development of rickets, but that it is the disproportion of the abundance of calcium and the small or perhaps even sufficient amount of phosphorus in the diet, together with the absence of »antirachitic vitamin«, that causes rickets. They are further confirmed in this impression by an experiment where calcium was excluded from the diet (which was beforehand poor in phosphorus and vitamin), and here they found a pathological state of the bones

not resembling rickets. Neither did they succeed in producing rickets when the content of phosphorus in the diet was increased. *P. G. Shipley* (225) is of the opinion that these conclusions which may be drawn from the experiments on rats, will be the same for rickets in children. But as regards the various diets of *McCollum* it must be said that even if they may be suited for examining the influence of certain experimental conditions upon the state of the organism of the experimental animal, this state being caused by the experimental diet, it is very uncertain whether they will be able to delimit and prove what is the principal aetiologic factor in the development of rickets. Too many inhibitory factors will be found in the diet. It also appears from the experiments that either the animals grow very little or more often they lose weight during the experiment, some even became paralysed in the posterior limbs, they often grew emaciated and failed to consume food. In many of the cases »the line test«, introduced by *McCollum* and his co-workers, has been employed.*)

They often use it as the only diagnostic to ascertain whether a commencing recovery of the rickety phenomena produced has occurred. This test, which *McCollum* himself points out is unreliable and influenced by numerous other factors, for instance hunger, is absolutely insufficient to determine whether or not commencing recovery has been produced. As a matter of fact, this uncertainty of the line test has subsequently made *McCollum* propose several other rachitogenic diets with subminimal amounts of A-vitamin in the form of butter and of a more adequate composition. To this must be added the very important fact that at any rate some of the experimental results derived from animals which had an increased content of phosphate in the diet and whose bones are described as normal, not rickety, are hardly correct, as the photographic reproductions do not show normal bone, as *Korenchevsky* has, in fact, pointed out,

*) This test is made by cutting the bone in the longitudinal axis, and immersing it in an AgNO_3 solution under radiation of light. The tissue containing calcium will then become black, while the uncalcified cartilage matrix and osteoid tissue are not blackened.

but osteoporosis. This faulty observation is due to the fact that it may be difficult, and requires a good deal of practice, to distinguish between osteoporosis and the normal bone of a rat; therefore, in such experiments, chemical examination of the bone should be made simultaneously.

If, on the other hand, certain growth-stimulating articles of food (5 per cent of butter) were added to the diet, the state of marasmus was retarded, and the rachitic changes became more marked. If they added 2 per cent of cod-liver oil to the diet, they could not prove any pathological changes. These last observations did not cause any change in their view of experimental rickets. As already mentioned, they have, however, recently proposed four new forms of diet to produce experimental rickets (168), viz. diets Nos. 4025, 4026, 4033, and 4034, and these have 5 per cent of butter-fat added. With these regimes they think they can better guard themselves against false deductions from the »line test«.

Sherman & Pappenheimer's (224) rachitogenic diet No. 84 consisted of patent flour 95 per cent, calcium lactate 3 per cent, and sodium chloride 2 per cent. This diet is obviously very deficient, not only as regards protein, fat, and salts, but also in regard to all three vitamins. These investigators, too, lay particular stress on the low content of phosphorus. And *Pappenheimer, McCann, and Zucker* and *Hess* showed that this diet could not prevent rickets even with an addition of 0.2 gm of butter daily, while by administering »Harris yeast-vitamin«, often combined with an addition of casein, they can prevent the development of rickets. The authors think this effect is due to the high phosphorus content of the yeast.

Korenchevsky made some rational experiments with an adequate diet as poor in phosphorus as possible, but otherwise rich in A-vitamin. No changes of the skeleton could be shown on a diet like this, at any rate not rickets, while animals fed on a diet free from A-vitamin and poor in phosphorus did not show essen-

tially more pronounced rickety changes than animals on his diet free from A-vitamin.

Already the experiments of *Schmorl* and *Heubner* were performed on a diet which was not only deficient in phosphorus, but as we can see now, also deficient in all vitamins. The changes of the skeleton thus produced are described as osteoporosis, and in some cases as changes similar to those of *Barlow's* disease (scorbut). The descriptions do not much resemble *Barlow's* disease, but rather osteoporosis, as the presence of osteoid tissue is mentioned.

From the experiments it appears that rickets cannot be produced on a diet merely deficient in A-vitamin, but that, according to the observations of *Shipley*, *McCollum* and others, it is necessary that the diet is poor in phosphorus, and rich in calcium, and at the same time secures a certain growth of the animals.

An attempt was therefore made to compose a more synthetic diet more in accordance with the physiological demands, and in which it would be possible to examine the importance of one factor separately. Such a diet free from A-vitamin was composed by *Hess*, *McCann* & *Pappenheimer*:

casein (purified)	21 gm.
rice starch	57 gm
crisco (A-vitamin-free fat)	17 gm
salt mixture	5 gm
yeast extract	0.06 gm

The salt mixture was kept as near as possible to the composition and the ash content in dog's milk and consisted of: NaCl 5.19, Mg SO₄ 7.98, NaH₂PO₄ 10.41, K₂HPO₄ 28.62, Ca (H₂PO₄)₂, H₂O 16.20. Calcium lactate 39.0, and ferricitrate 3.54. On this diet they, as well as numerous other investigators, thus *Glanzmann* & *Sieffert* (69), found that the experimental animals (young rats) after two months stopped gaining in weight, and then showed loss of weight, xerophthalmia, and death.

Hess, *McCann*, and *Pappenheimer* maintain that neither histologically nor radiographically did they find symptoms of

rickets. The skeleton was in all respects normal. The cartilage proliferation zone in strict contrast to rickets, showed a pronounced cessation of growth often showing only the height of 2—3 cartilage cells. This zone was intensively calcified. No osteoid tissue was found, though a certain lack of active osteogenesis, but no signs of rickets. On a similar diet *Glanzmann & Sieffert* could only confirm these observations.

And when it has been maintained by various investigators that the reason why rickets cannot be proved after such a diet, is that the experiment has covered such a long time that the rickets produced has disappeared again, is cured, *Glanzmann & Sieffert* on the other hand maintain that rickets has never been observed on such a diet, neither was that paralysis of the hind extremities observed which is typical of rickets in rats.

These paralyzes are not caused by rickets, but are due to the absence of B-vitamin in the diet.

Though *Glanzmann* himself maintains that rickets is most likely developed by the presence of the growth-promoting factor A, in the diet, that lack of this produces retarded growth, it never occurs either to him or the numerous other investigators that the histological picture of the skeleton of animals arrested in growth, cannot be called normal in all respects, on the contrary, one should try to look at the two diseases from the same angle.

Again, this is due to the investigators who have kept to the clinical observations and to experiments which, seen from a certain angle, may be used as an argument against the assumption of one common factor.

When thus *C. E. Bloch* (16, 17, 18) in his thorough investigations of xerophthalmia in children finds that only 12 out of 77 show slight degrees of rickets, this has been used as a decisive proof that the absence of the growth factor-A is of no importance for the cause of rickets. And as *Bloch* by treating rickety children with 2×15 gm of cream daily (and cream is supposed to be rich in the growth factor-A) could not see the effect on rickets, but when administering 2×5 gm of cod-liver oil observed it, this observation together with experimental experience

has been used as a proof that the growth factor and the »antirachitic principle« are not a unity.

When *Mc Collum* and Co-workers maintain that the more essential part of their rachitogenic diet is just the great surplus of CaCO_3 , then this is to some extent supported by the experiments on humans. It is known that a greater administration of CaCO_3 to humans on animal diet may cause a considerable rise in the phosphorus excretion with faeces, and hence it is inferred that the organism may in this manner be deprived of phosphoric acid. Observations similar to these have been made by *Czerny* and *Keller* with a diet of cow's milk, therefore they maintain that the content of calcium carbonate in the milk is just the reason why there is a smaller resorption of calcium on a diet of cow's milk, as this will be excreted unresorbed with the faeces, bound as calcium phosphate.

This is supposed to be in accordance with the recent numerous investigations of the content of Ca and P in the blood.

Thus *Howland* and *Kramer*, *Mariott & Howland* (164), *György*, *Frans Faber* (53, 54) and others all find a lower content of phosphorus in the blood in progressive rickets, (about 6 mgm per cent P), while the content of calcium is most frequently normal (about 10.1 mgm per cent), but also the calcium content is reduced, if the child is suffering from tetany.

Rickets and the Relation to Glands with Internal Secretion.

An increased excretion of calcium phosphate and a reduced phosphorus content in the blood having thus been proved in rickets, it has been thought (*E. Freudenberg & P. György* (59, 60), *Hogdson* (111)) that the organism is deprived of some of its content of alkali, by which arises an acidosis. This is supposed to cause an increased quantity of acid phosphates (more mono- than di-phosphate) in the urine from rickety children. This acidosis is thought by the Heidelberg school, (*Moro*, *Freudenberg*, *György*, and *Vollmer*) to be caused by diseases of the

glands with internal secretion, either a single one or several. They have examined the excretion of acids and phosphates in the urine after administration of various preparations of hormones, and they are of the opinion that they are able to divide these into those acting alkalotically (which effect an increase of metabolism) and those acting acidotically (which reduce the metabolism) the latter are supposed to be effective in rickets, the former in tetany. This is supposed to explain why the phosphate ions of the blood are diminished in rickets, since a functional predominance of hormones acting acidotically (parathyroidin) is supposed to retard the binding of calcium to the tissue in rickets.

It has never been possible to prove any change of the hydrogen-ion exponent of the blood, neither has any ketosis been proved. To this must be added that the acidity degree of the urine is too much influenced by bacteria, fermentation, standing over, delay in vesica etc.

To the glands with internal secretion has been attached an importance for the aetiology of rickets, to which no experiment entitles them. It is especially *Erdheim* who thought he was able to prove an enlargement of the parathyroid glands in rickets. In tetany *G. Herxheimer* (88a, 88b), and *Erdheim*, and *Haberfeld* (79a) have, on the contrary, found that the parathyroid glands had diminished and that hemorrhages and atrophy of the epithelial cords may be found. *Ethel M. Luce* (154) examined the parathyroid glands as well as the thyreoidea in rats fed on, respectively, a diet free from A-vitamin, poor in calcium and lacking both elements, and a diet absolutely free from salts. She thought she was able to prove some hyperplasia of the parathyroids in animals on a diet poor in calcium, and now and then in animals on a diet free from A-vitamin, but she did not find any changes of the thyroid gland. When *Margarete M. Kunde & L. Williams* (148) mention that they have found changes of bones similar to those in rickets, even xerophthalmia, in young rats which got an adequate diet, but were thyroidectomized, this is no doubt the consequence of the growth retardation which thyroidectomy causes in young animals. *Jovane & Vaglio*, *Hohlbaum*, *Reuss & Leopold*, *Frederick S. Hammett* (80 a) and *Ko-*

renchevsky (142, 143) have not been able to attach any importance to the parathyreoid glands, as regards the aetiology or pathogeny of rickets. *Korenchevsky* says that parathyreoidectomy does not cause any influence on the skeleton of rats fed on normal or rachitogenic diets. Rickets is most surely produced on a diet free from A-vitamin and poor in calcium. Only the difference in age is decisive for rickets or osteomalacia. Neither did *Korenchevsky* find any change of the skeleton after castration.

Later *Freudenberg & Gyorgy* (61) maintain that they only suppose that the glands with internal secretion influence the balance of acids and alkali, and thus the mineral metabolism, for which reason they only suppose an indirect influence on the rickety disturbances of the metabolism. They do not expect any specificity from hormon therapy in rickets.

Rickets and Light.

Already in ancient times it was observed that the climate played an important rôle in the incidence of rickets, and a certain importance was especially attributed to the lack of sunlight. It was observed that rickets was developed during the winter and spring months in the dark time of the year, while it was cured spontaneously in the summer months, and was only seldom found in September and October (*Kassowitz*, *Schmorl*, *Marfan* and others). Rickets is rare in sunny, warm countries.

Diesing (40) maintains that the insufficient light of the Northern summer plays a great rôle for the incidence of rickets, and puts forward the theory that light causes the formation of two pigments in stratum germinativum. One of these, ferriferous haemosiderin, is supposed to form the serum pigment, and contain iron and calcium and be closely related to lipochrom, and so contribute to the formation of bone by yielding calcium to the bone marrow. *Maffei* and *Feer* had observed that rickets was very rare in the High Alps, but frequent in the valleys, which

the sun only reaches a few hours of the day. *Hutchison* (126) had observed that amongst the poorer classes in India, rickety children were never found. These children were often artificially fed, but were exposed to fresh air and sunlight for several hours of the day, while children amongst the well-to-do people, who lived in accordance with the laws of *Purdah*, were often rachitic. The young mothers of these classes are always kept in dark rooms and most frequently suckle their children, nor do the children get any fresh air and therefore they often show rickets.

Furthermore *Chick*, *Dalyell*, *Hume*, *Henderson Smith* (25) and *Weinberger* (25 a) have shown that rickets is not produced in the summer on a diet which would cause rickets during the winter. *E. M. Luce* (155, 156) has shown that the strongest anti-rachitic effect of milk is reached by cows bred in light and on fresh green-stuff, without it being decided whether the light or the green-stuff is the decisive factor. *Eva Sopp* (235) also shows that an addition of 1 c.c. of milk to an A-vitamin-free diet causes an obvious difference in the growth of young rats, according as the milk is spring, summer, or autumn milk.

Hess has shown that white non-pigmented rats can be protected against rickets by exposure to light, while the pigmented black rats got rickets (*A. F. Hess & L. R. de Buys* (90)).

This theory is further based on the fact that rickets is of specially severe degrees in the highly pigmented Negro children in New York. Rickets has also been observed in breast-fed children in spite of routine employment of cod-liver oil (2.5 gm $\times$ 3 daily) (*L. R. de Buys & L. v. Meysenbug* (22)).

But the theory is especially based on the experiments on children with rickets made by *Huldschinsky* (121, 122). He showed that radiation from mercury-vapor quartz lamps on children suffering from rickets caused the rickety symptoms to diminish, clinically as well as in radiographs. *Unger & Hess* (105, 106) have reached results similar to these by exposure to sunlight, without changing the diet. Improvement was seen after 3—4 weeks. *Hess* showed that exposure to X-rays was ineffective and that the effective rays are the ultra-violet rays lying around 290 $\mu\mu$. *Poul Schultzer & Carl Sonne* (222) find the effective rays between 280 and 400 $\mu\mu$.

Hess thinks that radiation produces certain chemical substances in the skin and that these are resorbed into the blood and here act antirachitically. *Hess* also proved that the phosphorus content of the blood serum varies with the seasons, is lowest in December to January, increases suddenly in May and is at its height at midsummer.

Later numerous experiments have been made trying to show that rats on the rachitogenic diets of *McCullum* and *Pappenheimer* are prevented from getting rickets by being exposed to radiation (determined by histological, »line test«, and X-ray examination of the bones, but seldom by chemical bone analyses, and special histological examination). The first experiments even show that the growth of rats may be advanced by radiation from the mercury-vapor quartz lamp, nay, that even the air from this is sufficient (*E. M. Hume & H. H. Smith* (125)). This has later been disproved (*F. M. Webster & L. Hill* (265), *Hess* (89), *E. Walman & M. Vagliano* (264), *G. F. Powers, E. A. Park & N. Simmonds* (197), *Poul Schultzer & Carl Sonne* (222)).

Some of the most instructive experiments have been carried out by *Poul Schultzer* (220) (221) (221 a). He made an extensive series of investigations on the retention of P and Ca in young rats on *McCullum's* diet No. 3143, by determining the content of P and Ca in the urine and faeces. He examined this during five periods of four days each, in all an experimental period of 20 days, during which the rats got *McCullum's* diet No. 3143. During this time the retention of P and Ca slackened down. If the animals got an addition of cod-liver oil, or radiation with ultraviolet rays, or an addition of phosphate, he found that the retention of Ca and P increased in the last half of the experimental period. But while cod-liver oil and light treatment caused 20—30 per cent of the resorbed Ca-content to be excreted with the urine and 70—60 per cent with the faeces, only 5—6 per cent excretion with the urine was found in animals with an addition of phosphate, while the Ca-excretion with the faeces had increased.

Serum Ca and serum P also increased with an addition of cod-liver oil and with radiation with ultra-violet rays, thus *Schultzer* speaks of hypercalcaemia, while the addition of phos-

phate made serum P increase, but not serum Ca. He therefore assumes that cod-liver oil and ultra-violet rays have the same effect on experimental rickets, and that this effect is different from the »recovering effect« of an addition of phosphate. *Schultzer* finds that radiated, previously ineffective, oils, when given as an addition to the rachitogenic diet, have the same effect as cod-liver oil and radiation on rickets.

The diagnoses were made by means of radiographs, autopsy, and determination of blood Ca and blood P.

No explanation is given of why a content of serum P of 6 mg per cent is in some experiments regarded as normal, while in other experiments it is regarded as a decreased content.

Any histological examination or chemical bone analysis has not, unfortunately, been undertaken, nor is the growth and weight of the animals discussed. Add to this that the period of observation is only 20 days, that is to say, that if animals with an addition of cod-liver oil have grown well, and animals exposed to radiation have perhaps also shown some growth, the short period of investigation does not furnish any guarantee that growth is secured in the latter group. The possibility is obvious that the radiation has caused a rapid resorption of the spare supply of vitamin in the animal, and that this has for a short period caused the Ca and P retention to be increased, as well as serum P and Ca. It shows that the radiation with light has caused no synthesis or specific protection against rickets, but only a more rapid resorption of the available reserves.

John McAskill Henderson (88) has investigated the influence of light from a carbon arc lamp on guinea-pigs and found the excretion of Ca and P with the urine increased during radiation, actually as well as relatively, as compared with the excretion with the faeces, without the animal gaining in weight. Therefore he assumes that the retention does not necessarily involve any stimulus of the weight in general.

From all these experiments and observations it is evident that lack of light cannot be regarded as the causal factor in the incidence of rickets. Partly rickets is very rare in countries poor in light such as Greenland, Norway, Iceland, and the Hebrides. Rickets has been observed in hospitals for tuberculous diseases,

though the children here are exposed to as much sunlight and fresh air as circumstances will allow.

The clinical experience (*Marfan, Dorencourt & Turquety* (163), *Frans Faber* (53, 54)), showing that treatment with light of rickets in children causes the clinical, radiographical, and serologic symptoms to diminish, and may even cure rickets, by no means warrants the conclusion *ex juvantibus* that the lack of light is the primary aetiological factor; this has not been proved.

From experiments on rats it appears very clearly that it is only possible to produce rickets in the animals when they are kept in darkness, and other factors, namely the relative deficiency of phosphorus and A-vitamin in the American forms of diet, make their influence felt simultaneously. If $\frac{1}{7}$ of the calcium lactate is replaced by 0.4 per cent of K_2HPO_4 , e. g. by adding to the 86 mgm P of the diet No. 84 of *Sherman—Pappenheimer* a further 75 mgm (i. e. together 161 mgm of phosphorus per 100 gm of diet), then it is not possible to produce rickets even if the animals are kept in darkness, while animals under similar conditions but exposed to the influence of light escaped rickets (*Hess, Unger & Pappenheimer* (108), likewise *Powers, Park, Shipley, McCollum & Simmonds* (198) and *P. Christou* (26 a)).

Add to this that it has never been possible to produce growth of the animals, even in spite of treatment with light. Further it has been assumed by various investigators (*P. Reyher & M. Schmaucks* (202)) that an effect can only be obtained by radiation with ultra-violet rays when the children get plenty of A-vitamin at the same time. As a matter of fact, the children treated with light are admitted into hospitals, and there naturally have the benefit of better nursing and better food. *Hermann Vollmer* (260) made observations on rickets in twins simultaneously, one being kept on a diet rather poor in vitamin, and exposed to light for five weeks, while the other after the same regime for 17 days stopped with the treatment with light, but instead got a diet rich in vitamin. Even if *Vollmer* asserts that the first child improved quicker than the other one, it is not

convincing that the treatment with light has been superior to the treatment with vitamins, as information as to the weight is lacking, and the children have only been observed for five weeks. Nothing is mentioned about the utilizing of the diet, and the first child has furthermore been exposed to the possible effect of part inanition on the rachitic bone changes. Already the first X-ray-photo very likely shows commencing calcification.

It is strange that the treatments with light and cod-liver oil on many points show an apparently similar influence. It has been supposed that during the katalytic influence of the light the organism was able to synthetisise the antirachitic factor (the A-vitamin and the antirachitic factor must then be thought of as not identical). The antirachitic factor must then be supposed to neutralize the possible deficiency of phosphorus in the diet and increase serum P. The rays of light being only slightly penetrating, it must be supposed that the effect took place in the skin. It might then be thought that the content of cholesterol in the skin was synthetisised to antirachitic vitamin (*A. F. Hess & Mildred Weinstock* (92)).

The deficient growth of the rats treated with light seems to refute this. Now and then it is seen that the weight may increase a little during the first 3—4 weeks, but during the 5th to 7th week the weight becomes stationary, and then goes down rapidly.

D. I. Macht, F. K. Bell & C. F. Elvers (158) have shown that the ultra-violet rays are able to penetrate 2 mm or more into the skin of living rabbits, dogs, and cats.

Several investigators are of the opinion that by treatment with light certain processes of demolition take place. *A. Eckstein* (48) undertook histological investigations of bones from rats with experimental rickets, and examined the effect of radiation with light on the bones. He used *McCollum's* diet No. 3142 (with 5 per cent of butter) but used 3 per cent of CaCl_2 instead of CaCO_3 . He had the impression that radiated animals were alive a little longer than the non-radiated, but in both cases the animals developed keratomalacia. He mentions that a normal thorax and normal epiphyseal lines are found in

radiated animals, he says a little osteoid tissue is found, however.

Therefore it is surprising that he calls the bones normal, after the description it must be maintained that it is a case of osteoporosis with some osteoid tissue, so much the more since the bone growth has ceased, as the animals developed keratomalacia.

Later *Eckstein* (49) asserts that the conditions associated with rickets in rats cannot explain rickets in humans. He thinks »a disposition of age« is present in rats, and asserts that the effect of treatment with light is probably due to a resorption of cells in the organism, caused by this treatment, and that the disproportionate ratio of Ca and P in the diet is hereby neutralized.

L. P. Pincussen & K. Momferrato Floros (192) show that a heavier demolition of nuclein acid takes place after the radiation than before, and phosphoric acid is thereby liberated.

Whether it is possible that this phosphoric acid can compensate a possible deficiency of phosphorus in the diet and — if this is the cause of rickets — cure the disease, it is difficult to prove.

It is more probable that all these circumstances must be taken as a sign of an augmented metabolism. Such an increase of the basal metabolism during radiation with ultra-violet rays is supposed by *Otto Kestner* (137). Upon examination of two normal children and a child with simple goitre *Margarete E. Fries* (67) could not, however, prove any appreciable difference in the basal metabolism after radiation. She thinks that a prolonged increase is not general, but perhaps possible in the case of certain children.

It is hardly possible to undertake investigations of the respiratory metabolism in infants under physiological conditions, as it is difficult to keep the infants in repose and thus be able to judge of the basal metabolism, and it is the same with animals. *Carl Sonne* (234) has published a series of experiments on the influence of light on the oxygen metabolism in meal-worm pupae and has shown that the oxygen metabolism increased

(about 15 per cent) during longer periods after radiation with ultra-violet rays. The warming, yellow rays had no effect. But probably the technical method was not unimpeachable.

It might be supposed that an augmentation of the metabolism in the rickety organism by radiation would cause a resorption of such tissue in the organism, which also under other circumstances, when own tissue is resorbed, primarily vanishes, and thus may be supposed to be less important for life. In this connection we might especially think of the uncalcified cartilage matrix and the osteoid tissue, and perhaps just in this find the right explanation of that phenomenon which *McCollum* and others characterize as »healing by starvation«.

That inanition causes metabolic processes which cannot quite simply be explained by merely examining the fate of the individual substances in the organism, is evident by the investigations of *E. P. Cathcart* (24) on the P_2O_5 excretion with the urine in a cat on inanition. He finds the phosphoric acid excretion reduced.

Further, the increase of the phosphorus content of the blood which can be shown when rickets is treated with cod-liver oil, is hardly so exclusively associated with the phenomenon of recovery that we can, on that account, draw the conclusion that starvation and radiation also have a healing effect, because the phosphorus content of the blood is also increased thereby.

This view, then, should be brought to bear on the effect of the treatment by *Jundell* (132, 133), *Monrad*, and *Czerny* of rickets with relative inanition (60—70 calories per kilo per diem).

As a rule *Jundell* uses a diet as rich in vitamin as possible. Even if the administration of calories to children is kept low, most of them have cod-liver oil as well, whether or not they are treated with light.

The little we know of the biological effects of radiation with light points to regressive phenomena.

II.

THE AUTHOR'S OWN EXPERIMENTS.

When I shall, in the following, try to contribute to the solution of the question of the aetiology of rickets, the first question to be answered will be: how is experimental rickets produced?

What experimental conditions are required to produce experimental rickets?

Can the experiment be thus arranged that the effect of one condition at a time can be examined?

Can the rickets thus produced be assumed to be identical with human rickets?

From all previous experiments it appears that experimental rickets is chiefly produced by an experimental regime, which is under the influence of several factors simultaneously. Thus the various rachitogenic diets for rats of *McCollum* and *Pappenheimer* are deficient in phosphorus, while there is, at the same time, a very abundant supply of CaCO_3 or CaCl_2 — a fact to which the authors attach much weight — and the diet furthermore contains a rather accidentally and incalculably varying amount of A-vitamin. The diet is likewise, judging from several experiments, deficient in B-vitamin, as in many of the experiments paralysis of the hind limbs have occurred. Add to this that the animals as a rule do not grow, but mostly come to a stand-still or lose weight during the 5—8 experimental weeks.

The experimental conditions must therefore be supposed to be different from the conditions causing rickets in children.

Furthermore the histological changes of the bones do not appear to be like the rachitic changes in the skeleton of children, as the bones seem to be very osteoporotic. Simultaneously a considerable cartilage cell accumulation takes place in the zone corresponding to the provisional calcification zone including the proliferation zone, while the calcification has absolutely ceased. Of this later.

It is particularly at the English institutes, and here especially in the experiments of *Korenchevsky* (140), that an attempt has been made to use a synthetic diet sufficient for the normal growth of rats, their fertility, and ability to rear young.

In such a diet, in which it will be possible to experiment with the absence of one factor at a time, the composition will be the following :

purified caseinogen	18 gm
rice starch	52 »
fat	15 »
marmite (a yeast preparation)	5 »
orange juice	5 c.c.
salt mixture	5 gm

(the salt mixture is that mentioned at page 45, given by *McCollum*).

For animals under normal conditions the fat consisted of 12.5 gm of butter + 2.5 gm of cod-liver oil. For animals on a diet devoid of A-vitamin the fat consisted of cotton-seed oil 15 gm. This diet contains 0.256 per cent of Ca and 0.5 per cent of P. This A-vitamin-free diet probably contains a slight amount of A-vitamin, partly not yet destroyed traces in the purified casein, partly a slight amount in the cotton-seed oil, and finally a very small amount in the orange juice.

When this diet was administered to young rats, *Korenchevsky* as well as numerous other investigators (*Hess, McCann & Pappenheimer*) found that the changes produced in the skeleton were mostly osteoporosis, but in some cases was found a picture very similar to rickets, though with distinct signs of osteoporosis too. The increase of weight from the commencement to the termination was small. If the experiment lasted for 6 weeks or more, there was cessation of growth and often xerophthalmia.

As *Korenchevsky* in the histological picture finds a smooth transition from rickets to osteoporosis, and even finds rickets and osteoporosis in the same bone, he asserts that rickets is present in latent form in the osteoporotic bones.

He assumes this to be phenomena analogous to the latent rickets met with in cachectic children, and the hyperplastic rickets met with in better-nourished children. He further assumes that all the conditions influencing the growth of tissue will, when absent, as in starvation, wasting diseases, absence of vitamins etc., contribute to the development of osteoporosis.

But then *Korenchevsky* asserts that »the mechanism of development of this skeleton disorder is quite distinct from rickets, since the cause of rickets lies in the disturbance of calcium metabolism, while osteoporosis is caused either by delay in the formations of new bone or in the increased destruction of old« (Pag. 112).

»There was no essential difference between the figures for calcium retention of rats, grouped and arranged according to the severity of rickets.

Therefore from all the facts mentioned here I conclude that the antirachitic factor operates by influencing the capacity of the animal to assimilate calcium.« (Page 115).

He thinks this is due to the fact that the »antirachitic vitamin« either causes better calcium resorption from the intestinal canal or that, as an »amboceptor« it causes better deposition of the calcium in the tissues, or that it operates in both manners. In calcium starvation he has, however, seen pictures similar to those of an A-vitamin-free diet, so he supposes it may be a case of deficient resorption from the intestine.

But *Korenchevsky* also asserts that the age of the animal when subjected to experiment (less than 5—6 weeks), and certain deficiencies in the diet of the mother animal during lactation and gravidity, are decisive factors for the development of pronounced rickety changes in the experimental animal.

He is most successful in producing experimental rickets when the diet of the experimental animal is deficient both in A-vitamin and in calcium. But this causes the growth of the animals to be still more deficient.

As a diet for my experiments with rats I used a pure synthetic diet, used during many years at the Hygienic Institute of the University.

This diet is composed of

purified caseinogen	18 per cent
purified rice starch	54 per cent
dry, autolyced top yeast	5 per cent
salt mixture	5 per cent
purified agar-agar	3 per cent
fat	15 per cent.

The caseinogen is purified by extraction in 96 per cent alcohol for 24 hours, the alcohol boils away, continued extraction in ether for 24 hours. Now the caseinogen is dried and heated on flat trays for 24 hours at 100° Celsius during sufficient supply of air.

The rice starch was mostly treated with 96 per cent alcohol for 24 hours, then filtered, dried, and crushed.

The surface yeast was autolyced for 24 hours at 37° Celsius.

Agar-agar was pulverized and purified by washing out in 96 per cent alcohol.

The salt mixture was varied according to the conditions whose influence it was intended to examine.

The salt mixture which proved adequate for normal growth, development of bone and other biological conditions in rats was (after *McCollum* No. 185)

Salt Mixture I.

sodium chloride	10.38 gm
magnesium sulphate	32.80 »
sodium acid phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)	20.82 »
potassium phosphate (K_2HPO_4)	57.24 »
calcium phosphate ($\text{Ca} (\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$)	32.40 »
calcium lactate	78.00 »
ferrie citrate	7.08 »

Fat for the control animals, for which normal conditions of growth are required, is 15 per cent of melted, filtered, water-free butter-fat.

When an A-vitamin-free diet was desired, the fat was lard. This was melted from pork, and was then heated to 102° C. for 24 hours on flat trays in a current of air, later it was modified

so that the melted lard was heated to 100° C. and for 10 hours it was bubbled through with air.

Such a diet was partly by calculation, partly by my analyses, found to contain 0.25 per cent of Ca, and 0.5 per cent of P.

The diet was carefully mixed and stirred with distilled water to a thick paste. Furthermore the animals could drink distilled water from a vessel ad libitum. The experimental animals lived in a thermostat at 20° C. with absolute exclusion of daylight. Each animal lived in its own cage. It was made impossible for the experimental animals to eat their own excrements.

The diet with 15 per cent of butter has proved quite sufficient for rats, it ensures normal growth and development, fertility, and the ability to rear young.

I have good grounds for supposing that our A-vitamin-free diet is as short of A-vitamin as is possible. The purification of the caseinogen is very intensive (*Korenchewsky* only calls his: purified casein, and there is reason to suppose that the purification has not been as thorough as ours).

The agar-agar, yeast, and starch were very carefully purified, and the oxidated, heated lard was totally devoid of A-vitamin. After the experiments of *Fridericia* (63) there is even reason to suppose that the oxidised lard has acquired the ability of destroying any A-vitamin which might still be found in the casein, agar-agar, and yeast.

As fat, *Korenchewsky* used cotton-seed oil, and this probably contains slight traces of A-vitamin, as it appears to give a slower cessation of growth than my A-free diet. I also found that 15 per cent of olive oil, which is also considered to be devoid of A-vitamin, causes a slower and less complete cessation of growth than the diet with 15 per cent of oxidated lard.

Below I shall schematically arrange the chief forms of diet used in this work.

1) Normal diet (*N-diet*): a diet which in all respects is adequate for rats. It is mentioned on page 60, contains salt mixture I (page 60).

2) A-vitamin-free diet (*minus A-diet*): a diet with an ade-

quate salt content and composed just like the N-diet, only with 15 per cent of oxidated lard instead of butter fat. The diet contains Ca, 0.25 per cent, P, 0.5 per cent.

3) A-vitamin-free diet with insufficient calcium content (*minus A-, minus Ca-diet*) composed like minus A-diet but with

Salt Mixture II.

NaCl	5.19 gm
MgSO ₄ , 7H ₂ O	16.4 »
Na ₂ HPO ₄	27.91 »
K ₂ HPO ₄	28.62 »
ferri citrate	3.54 »
Na HCO ₃	21.2 »

The diet contains Ca, 0.04 per cent, P, 0.55 per cent.

4) A-vitamin-free diet with insufficient phosphorus content (*minus A-, minus P-diet*) composed like minus A-diet, but with

Salt Mixture III.

NaCl	9.6 mg
MgSO ₄ , 7H ₂ O	16.4 »
KCl	24.4 »
calcium lactate	57.5 »
ferri citrate	3.5 »

The diet contains Ca, 0.25 per cent, P, 0.2 per cent.

The Effect on Experimental Rats of Feeding with a Diet totally devoid of A-vitamin but otherwise adequate.

As mentioned above, similar experiments have been undertaken by numerous other investigators who, in the great majority of cases, have found that the skeleton changes thus produced are osteoporosis, but *Korenchersky*, whose experimental diet I have followed in all essentials, further found microscopically demonstrable rickets in a greater or less number of cases. As is mentioned, it must be supposed that *Korenchersky's* diet still contains slight amounts of A-vitamin, partly in the orange juice, partly in the casein and in the cotton-seed oil.

As I consider the diet used by myself absolutely A-free, it will for that reason alone be of interest to examine the effect of this diet on the experimental animals, so much the more as it is the basal diet in the more extensive of my experimental series.

The experimental animals were black and white-speckled rats as generally used at the Institute, a sub-species of *rattus norvegicus*.

The breeding animals lived in constantly heated cellars, at about 20° C., with diffused daylight entering from a rather large corner window. The diet of the animals was composed of oat, maize, brown-bread, and milk ad libitum. When they were pregnant they were put in their own box on the same diet. The young rats stayed with their mother and were subjected to experiment when about 30 days old.

When the young rats were put on this diet, about 30 days old, their weight was between 35—50 gm. They ate the diet for the first 3—4 weeks of the experiment, about 6—10 gm daily, after that their appetite decreased and in the following weeks they only ate 4—6 gm daily.

The weight increased, even though considerably slower than in animals receiving an optimal A-vitamin content in the diet. In some cases, however, the growth (C 24, C 26, C 28) for the first 2—3 weeks was as rapid as in normally nourished animals, but after the 3rd—4th week the increase of weight was either very slight or had ceased. Then during the 4th—5th—6th weeks the weight curves were stationary or showed loss of weight. The loss of weight may be very rapid the day before the occurrence of death.

In the 4th—5th week it becomes evident that the animal is ill. The fur bristles and grows rather thin, the hairs around the borders of the eyes come out, the borders »become hairless«. There will be a secretion in the corners of the eyes, this may be rather mucous, often haemorrhagic, later often purulent. Enophthalmus commences as described by *Eiler Holm* (112), *Fredericia & Holm* (66). Palpebrae stick together, later inflammation of the eyes with keratitis, wounds on cornea, hypopyon, panophthalmia supervenes, now and then with sequestratio lentis, anophthalmia. The animal becomes apathetic, and ema-

ciated, and does not clean itself, is overgrown with mites and lice, cachexy supervenes.

Upon autopsy, besides the diseases of the eyes, fractures of the teeth were often found and friable thin teeth, which were easily drawn, and showed a rather short root with a wide cavum dentis and thin substantia eburnea and often fissures in substantia adamantina and worn incisive faces.

In a great many of the cases were found fractures of the ribs in greater or less number. The bones were osteoporotic, which could already be seen macroscopically, as the marrow cavity was enlarged and compacta thin and fragile. In these animals were hardly found rickety rosary or swelling of the epiphysis in the bones of the limbs, but often more or less callus around the fractures.

The definitive mortal causes seem to be inflammation. The inflammation may be very differently localized and has previously always been understood as a direct consequence of the absence of A-vitamin, as it has been assumed that the power of resistance of the organism, its production of antigenes, its phagocytosis, and its production of opsonin, should have been reduced, owing to which the organism more easily succumbs to intercurrent infectious diseases.

These inflammations are sometimes pneumonia, often cystitis, with apparently ascending ureteritis, pyelo-nephritis, often cystitis calculosa with bleeding in vesica, retentio urinae, dilatatio ureteris and pelvis renis. Now and then is seen primary nefritis with great brindled kidneys.

Often inflammation of prostata and vesiculae seminales, often abscesses in the root of the tongue, inflammation in the salivary glands, inflammation in *Harder's* gland, further purulent otitis media and mastoiditis. Often numerous organs in the same animal are the seat of purulent inflammations.

The Technique of Examination.

Autopsy was made on all the animals from the experiments here described. Attention was especially directed to palpebrae, eyes, teeth,

tongue. Processus mastoideus was examined by trepanation from nasopharynx. The salivary glands, lungs, peritoneum, kidneys, ureteres, vesica, prostata, and vesiculae seminales were examined, further the thorax, costae, and columna. The right femur was dissected, freed from all soft tissue and ligaments. The left knee-joint with the lower half of femur and proximal half of tibia and fibula was removed to be used for the histological examination, together with costae and the costal cartilage on both sides of thorax.

Chemical examination of the right femur. This was dissected, freed as much as possible from all soft tissue, insertions of muscles, ligaments, and tendons, as quickly as possible. Femur was then weighed in the fresh state, dried in a kiln at 110°C . for 8–10 hours to constant weight, weighed; petroleum ether extraction of the fat for 48 hours, dried to constant weight, weighed, incinerated, the ash weighed; determination of the content of CaO and P in the ash.

The bone ash was dissolved according to the method of *Francis M. McCrudden* in $\frac{1}{2}$ –1 c.c. concentrated HCl. After complete dissolution 5 c.c. distilled water were added. After this neutralized with NH_4OH drop by drop until turn of the added indicator, methyl-red (pH:5). 10 drops of concentrated HCl were then added.

Ca was then precipitated by addition of 10 c.c. of a 2.5 per cent oxalic acid. Shaking for 10 minutes. Then 10 c.c. 3 per cent ammonia oxalate. Now there was a beginning precipitation of calcium oxalate, and 5 c.c. of a 20 per cent natrium acetate solution were added, then again shaking for 10 minutes and it stood till the next day.

Next day the calcium oxalate was filtered off, was washed with 0.5 per cent solution of ammonia oxalate and finally several times with distilled water (until the water is free from Cl^- , tried with AgNO_3).

The washed calcium oxalate was now poured into a retort. 5–10 c.c. of concentrated H_2SO_4 were added. Boiling for 10 minutes and then titration with $\frac{1}{10}$ -n solution of potassium permanganate from Bang's burette. The titration was carried out at 90 – 100°C .

To the filtrate from the precipitation of the calcium oxalate was added 5 c.c. of concentrated HNO_3 and it was vaporized until dry. Thereupon was employed the method created by *Poul Iversen* (130) and *Ejnar Lenstrup* (149) and later to some degree modified by *Margrethe Sørensen* (254).

To the vaporized filtrate was added 1 c.c. of concentrated HNO_3 and 20–30–50 c.c. of saturated solution of ammonia nitrate, heated and boiled. To the boiling solution was added 20–50 c.c. of a 10 per cent solution of ammonia molybdate. In this way the yellow ammonia-phosphorus-molybdate was precipitated. The retort was shaken for 10 minutes while the refrigeration took place slowly. It stood over till the next day.

Next day the ammonia-phosphorus-molybdate was filtered off on

hardened filter. The precipitate was washed with distilled cold water until the rinse water was neutral (lithmus). The whole of the precipitate was washed down into a half litre retort. From a burette was added $\frac{1}{10}$ -N NaOH in surplus. The amount of NaOH was noted. Now boiling for one hour on reticle. Refrigeration, addition of one drop of phenolphthalein, $\frac{1}{2}$ per cent alcoholic solution. Retitration with $\frac{1}{10}$ -N H_2SO_4 . From this the consumed amount of $\frac{1}{10}$ -N NaOH was calculated and by multiplication of this number of c.c. with the factor given by *Neumann*, P was found in mgm.

Histological Examination.

The bones were fixed in formaldehyde-alcohol 1—2 days; then partial decalcination in Müller's fluid in thermostat for 5—8 weeks. The fluid was changed 2—3 times. Now thorough washing for 24 hours, 70 per cent alcohol for 24 hours, 93 per cent alcohol for 24 hours, 99 per cent alcohol for 24 hours, Xylol for 2—3 hours, paraffin kiln for 3—4 hours (56° C). Imbedded in paraffin. The costae were cut about 6 μ thick, femur and tibia were cut 10—12 μ .

The cuttings were stained with potassium-alum-hematoxylin, no differentiation with HCl-alcohol. Staining with eosin or a solution of ammonia-carmin. Other cuttings were stained with *Schmorl's* thionin solution and differentiation with phosphorus-wolfram-acid.

All bones were furthermore, according to *Pommer's* method in some cuts treated with 3 per cent AgNO_3 -impregnation by daylight or electric light for 1—2 hours, washed out with distilled water. Reduction in 10 per cent $\text{Na}_2\text{S}_2\text{O}_3$ -solution for 2—3 minutes. Thorough washing in running water. Then staining with potassium-alum-hematoxylin and again washing for several hours. Staining with eosin.

In this way all calcified parts of the bone tissue and of the cartilage matrix were thoroughly blackened. The osteoid tissue was bright red, the nuclei were deep blue, and the unchanged cartilage matrix was blue. All tissue which was calcified, and the tissue which had been partly freed from calcium during the stay in Müller's fluid was plainly black, and was quite distinct from the osteoid tissue and the uncalcified cartilage matrix.

For more thorough examinations of the bone marrow *Romanowsky-Giemsa's* solution was used.

For practical reasons I had to desist from the determination of Ca and P in the blood. This will hardly be possible for the same investigator in view of the other examinations. The amount of blood which can be obtained from one rat for these experiments will partly be too small, often even too small for such an investigation, and partly it would be difficult to obtain blood from the dead experimental animals. Furthermore it is possible that death may cause processes of diffusion, which may influence the content of Ca and P in the blood.

The investigators who have undertaken such examinations of rats have always used an amount of blood composed of blood from several living or just killed rats under the same experimental conditions.

And to this must further be added a more theoretical objection: the fact that only relatively little is known about the factors influencing Ca and P in the blood. Numerous observations have already shown that we cannot from a reduced P content in the blood draw the conclusion that rickets is present. Further that the P content, if it has been reduced, increases rather regularly if the animal is starving, this being often more or less completely the case with animals on an A-vitamin-free diet. The estimation of the amount of Ca and P of the blood is inconstant in the different investigations and by the different investigators.

I shall now schematically arrange the animals on A-vitamin-free diet, as well as three animals (R 30, R 31, R 32) where the A-free fat in the diet consisted of 15 per cent olive oil. There are 26 animals from 13 different litters of rats, the animals having served as control animals in various series of experiments.

This offers the additional advantage that any differing conditions in a litter cannot lead to false conclusions as to this applying also to other litters.

My experiments have shown that the animals on this diet present a very constant picture.

The histological changes in these animals consist in 13 cases of pure osteoporosis, without any, even the slightest sign of rickets. Periosteum is seen very low, with a low cambium layer consisting of extended narrow cells. After this a very thin well calcified compacta. The marrow cavity dominates the greater part of the section of the bone. There are few, narrow spongy bone trabeculae, in certain parts of the bone none. The few spongy trabeculae which can be pointed out are calcified.

The marrow cavity presents a somewhat different appearance; of this later. The marrow cavity in some of the bones is extraordinarily hemorrhagic with wide blood vessels, slight fat tissue and around the blood vessels are found marrow cells. These are rather homogeneous, principally small myelocytes or normoblasts, only few megakaryocytes and numerous lymphocytes.

The cartilage in the epiphysis: the resting cartilage cells present nothing abnormal. In the proliferous zone are arranged only few cartilage cells in short columns, 4—6 cartilage cells in each column. The formation of columns is little dominating and the cartilage capsules small with only 1—2 cartilage cells in each capsule. It is also conspicuous that no real provisional calcification zone is found, as the cartilage finishes with a sharp cut line towards the marrow cavity. This »line« seems to be calcified, but, as mentioned, it is very narrow (the thickness as of one to two cartilage cells).

That which, upon exact observation, strikes one as the peculiar feature in these bones is the total cessation of growth in the cartilage, the lack of ossific direction columns towards the marrow cavity and certain phenomena in the formation of the marrow which, as we shall show later, must be apprehended as cessation of growth.

Thus, if attention is not directed towards these facts, it might be believed to be normal bone. The histological pictures often seem to have been understood in this way, or the explanation has even been given that there is a possibility of rickets having been present, caused by the A-free diet, but having again been cured, when the animal had starved during the last days of the experiment.

In twelve of the bones was besides found a picture which must be regarded as osteoporosis, but in several of the bones the cessation of growth is less pronounced, the cartilage proliferation zone is somewhat higher, some more cartilage cells lie in rows in the column zone. Near the limit towards the marrow cavity there is some calcification of the cartilage matrix, but no dissolution of the cartilage cell capsules is seen. The cartilage columns extend into the marrow cavity, either in arcs or somewhat irregularly, and here short rows or small isles of unopened cartilage cells resting on a thin border of calcified matrix are seen.

Further in some of these bones are seen few, very thin borders of osteoid tissue around the sparse spongy trabeculae. These slightly pronounced marks of rickets are mostly

found in costae, while femur and tibia in the same animal may show purely osteoporotic pictures.

For comparison I have picked out 7 animals, 4 of which had a quite similar diet, only the fat was melted butter fat, instead of lard, but in the case of the three animals (R 33, R 34, R 35) an unsaponifiable residuum from 30 c.c. of cod-liver oil had been dissolved in 150 gm of olive oil and thus utilized as 15 per cent fat in the diet. These animals proved normal in all respects.

Schematic View of the Content of Water, ASH, Ca and P in Right Femur from Rats on ÷ A-diet and Rats on N-diet respectively.

÷ A-diet.

No. of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fat free femur	of fresh femur	ash of dry fat free femur	of fat free femur	of fresh femur	
R 32	35	64	47	75	60.9	37.0	47.4	17.5	6.8	17.6	6.5	2.5	—
R 43	36	64	44	67	55.8	38.9	49.6	19.3	7.9	17.5	6.8	2.7	7.4
B 58	32	66	40	56	56.5	45.6	48.7	22.2	9.2	19.6	8.9	3.7	4.8
C 7	30	65	26	45	58.9	37.7	44.9	16.9	6.8	24.9	9.4	3.7	2.2
R 44	36	64	34	52	57.0	37.5	48.8	18.3	6.7	15.0	5.6	2.0	6.2
R 45	36	64	42	64	57.7	39.1	45.0	17.6	6.8	17.3	6.7	2.6	8.3
R 30	35	69	46	58	55.8	38.7	48.7	18.8	8.2	17.9	6.9	3.0	1.1
R 126	57	72	45	60	54.0	43.3	53.0	22.9	9.8	21.7	9.4	4.0	6.6
D 27	33	75	32	50	58.9	47.2	54.1	25.5	9.3	26.5	12.5	4.5	11.4
D 56	31	75	36	45	62.1	42.1	50.4	21.2	8.0	20.8	8.8	3.3	0.9
R 31	35	80	41	61	45.9	41.1	47.5	19.5	9.9	18.0	7.4	3.7	6.3
R 37	35	80	42	45	43.5	40.1	49.7	19.9	11.2	17.8	7.1	4.0	0.8
B 24	32	81	38	50	59.7	37.5	50.1	18.8	6.9	18.6	6.9	2.5	8.1
B 27	32	84	40	52	58.2	43.3	47.6	20.6	8.5	18.1	7.8	3.2	1.7
B 17	34	87	41	55	55.5	46.4	49.4	22.9	10.0	16.9	7.8	3.4	1.6
B 37	32	84	34	55	52.8	40.4	48.2	19.5	7.7	19.2	7.7	3.0	15.9
C 24	35	88	27	78	55.1	50.6	50.3	25.5	10.8	18.6	9.4	4.0	3.6
R 80	45	98	39	60	57.6	43.7	48.8	21.3	9.0	18.3	8.0	3.3	0.8
R 81	45	101	55	62	51.8	48.8	51.7	25.2	12.0	18.8	9.2	4.3	1.1
R 128	47	98	47	54	56.0	46.5	52.4	24.3	10.1	22.3	10.3	4.3	5.8
R 129	47	98	39	50	56.5	42.9	51.8	22.2	9.6	19.5	8.3	3.6	0.2
C 28	35	103	28	74	53.6	46.0	52.2	24.0	10.6	19.0	8.7	3.8	0.4
B 28	32	108	33	48	53.2	55.4	50.1	22.7	10.7	22.2	10.1	4.6	2.0

No. of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	'CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur			
R 127	47	112	51	67	58.0	45.1	52.7	23.7	9.9	20.2	9.2	3.8	0.2
D 8	34	126	31	81	56.6	46.6	53.0	24.6	10.6	17.3	8.0	3.4	0.5
D 31	31	165	37	68	55.4	42.5	51.6	21.9	9.7	22.9	9.7	4.3	0.6
<i>N-diet.</i>													
R 33	35	80	46	120	39.9	54.0	50.4	27.2	15.8	16.8	9.1	5.3	3.2
R 34	35	80	47	122	37.8	55.4	50.2	27.9	16.9	17.0	9.4	5.7	2.7
R 35	35	80	45	145	40.3	54.3	51.6	28.0	16.3	16.8	9.1	5.3	2.3
R 36	35	78	47	114	45.3	55.6	51.1	28.4	15.2	16.8	9.3	5.0	2.2
R 38	35	80	43	105	45.8	54.6	51.3	28.0	14.6	16.7	9.1	4.6	3.5
R 39	35	80	47	140	44.2	52.3	50.8	26.6	14.3	17.7	9.2	4.9	3.7
R 46	36	64	48	103	49.1	50.2	48.2	24.2	11.8	16.3	8.2	4.0	4.3
<i>Average of animals on ÷ A-diet.</i>													
in all													
	6	34	64	39	60	57.8	39.3		7.3			2.8	
	4	36	72	39	52	57.7	42.8		8.8			3.7	
	7	33	83	37	56	52.9	42.8		9.3			3.4	
	7	42	102	41	59	55.2	46.9		10.2			3.9	
on N-diet													
	6	35	80	45	124	42.2	54.3		15.5			5.1	

From this table it is evident that the water content decreases with the age of the animal, it is lowest in animals on ÷A-diet (55—60 per cent water in animals from 64 to 90 days old, about 55 per cent in animals from 100 days old and upwards), while in normally fed animals there is only found 50—40 per cent of water or a still lower water content. The ash content of the dry fat-free bone is a little lower in young animals than in old animals, is considerably lower in A-free animals than in normally fed animals. This would seem to show that the percentage proportion of organic uncalcified substance is greater in the bones of the animals fed on the A-free diet than in the bones of the normally fed animals, in spite of an essentially greater fat-content in the latter, which have even in advance been extracted with petroleum ether.

The fluctuation in these components from animals under

otherwise similar experimental conditions, may be explained by the following facts. First, no two animals are homologous, they differ in weight from the beginning, in spite of the same conditions they grow with varying rapidity, and as regards the animals on the A-free diet, the cessation of growth is not simultaneous, and not equally sudden, the loss of weight not equally rapid and violent. While some of the animals are early and much injured by infections which may produce fever, increased metabolism, and quicker loss of weight, other animals are less injured, and now and then have much fewer general symptoms.

Very emaciated animals will often show rather a large content of water for their age in the fresh bone, and a great content of ash in the dry fat-free bone, compared with animals which have suffered less violent loss of weight.

This must be due to a much smaller amount of organic substance (proteins) in the bones, apart from the inconsiderable content of fat and the great water content.

Furthermore it may have some influence whether the bones are taken for chemical examination shortly after the death or perhaps not until 24—48 hours after. The ratio between CaO and P in the bones is the same for animals fed on an A-free diet and normally fed animals, and constitutes the same percentage amount of the content of ash. But in conformity to the reduced content of minerals, the CaO and P content is considerably lower in the A-free animals than in the bone of the normally fed animals, calculated in relation to dry fat-free bone and especially in relation to fresh bone.

In animals which are much emaciated and have very hydrous bones and a slight content of fat, the ash percentage of the dried fat-free bone may be relatively high, which may further cause a relatively high percentage content of CaO calculated from dry fat-free bone, therefore this figure gives less illustrative information of the real content of CaO in the bone, as will be seen from the CaO percentage calculated from fresh bone.

No pronounced difference of the ash content is found in the bones showing total cessation of growth and those in which slight symptoms of rickets may be shown histologically. These

symptoms of rickets may be found in animals dead or killed young (60—70 days old) as well as in animals which have reached a higher age (100—130 days old) in spite of the A-free diet.

Thus, the experimental rickets produced on a totally A-free diet is partly little pronounced, in the main characterized by a considerable osteoporosis, partly the slight rickety symptoms are only present in half of the experimental animals.

As orientating experiments had already shown me this, my aim was to find out whether the A-free diet, when the Ca content in this diet was also reduced, would be able to produce more constant and more pronounced rickety changes.

Investigations on the Effect of $\div A$ - $\div Ca$ -Diet on Rats.

For these experiments were used 8 young rats of the same litter (28 days old). 4 of the animals were put on $\div A$ $\div Ca$ -diet, while 4 others had the same diet, but two of them (R 25, R 26) had 5 per cent cod-liver oil mixed in the diet, and the two remaining ones (R 23, R 27) had cod-liver oil ad libitum.

As it was to be expected, animals on this diet, which is not only deficient in the vitamin necessary for life, but also lacks the necessary mineral component, calcium, would grow even less than when fed on $\div A$ diet.

The weight of the animals remained stationary or even decreased during the 5—7 experimental weeks. The appetite was poor. The animals soon presented a suffering appearance. They were small, slight, and shrank from noise and exposure to light. Often their bodies or extremities trembled. Symptoms of xerophthalmia commenced after 5—6 weeks, and death occurred under the symptoms of cachexy.

Upon autopsy was found emaciation, keratomalacia, friable, thin, often fractured teeth. Fragile thin bones, often numerous fractures of the ribs with some callus around. Thickening of the borders of the rib cartilage and bending and irregularities of it.

Now and then abscess at the root of the tongue, cystopyelonephritis.

The animals which had cod-liver oil simultaneously grew slightly but steadily, the growth curve showed a steady rise, but lay considerably under the curve of animals with normal calcium content in the diet.

The autopsy of these animals showed thin fragile but solid bones no fractures and otherwise nothing abnormal in the organs.

Histologically the findings in animals on $\div A$ - $\div Ca$ -diet were pronounced osteoporosis with cessation of growth, but besides this the epiphyseal line presented certain differences from the low rather homogeneous cartilage formation found in the epiphyseal line in animals on $\div A$ -diet. In animals on $\div A$ - $\div Ca$ -diet was found a higher formation of non-differentiated, untransformed cartilage cells which in some places were found more or less irregularly preserved in the marrow cavity of the bone. These cartilage cells are rather small with preserved capsule, clear protoplasm, and some retracted nuclei. The matrix around this is entirely uncalcified. Spongy trabeculae are very rarely found and often surrounded by osteoclasts. In some places, amongst others on the inside of lamina compacta, osteoid tissue is found. The marrow near the epiphyseal line is transformed into fibrous, »fusiform-cell«-containing tissue.

This gives a picture which reminds one of rickets, but as the cessation of growth is the most obvious feature as also the atrophy of bone, it will be seen that the most conspicuous effect of this diet is osteoporosis.

In bones from animals which had cod-liver oil added to the diet, the bone tissue is also sparse. Growth phenomena are found in the proliferous zone, there is calcification in the provisional calcification zone; even if the formation of bone is rare, still these processes are normal in the direction lines of the ossification, and no osteoid tissue is found; the epiphyseal lines are straight.

Schematic View of the Content of Water, ASH, Ca and P in Right Femur from Rats on $\div A$ - $\div Ca$ -Diet.

No. of rat	Age in days at the		weight at the		H_2O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur	of fresh femur	of fresh femur	
R 22	28	58	33	30	70.9	27.8	39.0	10.8	3.1	24.5	*6.8	1.9	—
R 24	28	62	35	28	67.9	23.8	43.5	10.3	3.3	24.6	5.8	1.8	—
R 28	28	60	33	28	68.5	25.6	41.5	10.6	3.3	19.2	4.9	1.5	—
R 29	28	31	33	33	70.0	31.4	53.8	16.9	5.0	19.1	6.0	1.8	—
<i>$\div A$-$\div Ca$-diet plus cod-liver oil.</i>													
R 27	28	98	35	75	55.6	42.8	48.5	20.7	8.1	18.5	7.9	3.1	11.5
R 26	28	98	33	73	54.6	44.1	51.5	22.7	9.5	18.9	8.3	3.4	8.4
R 25	28	62	35	44	59.6	27.9	49.5	13.8	5.5	20.2	5.6	2.2	—
R 23	28	62	35	47	57.8	30.3	45.3	13.7	5.8	20.7	6.2	2.6	—

The chemical bone examination showed that $\div A$ - $\div Ca$ -fed animals have a considerable water content in the bones, thus animals 8—9 weeks old show as hydrous bones as when they were put into experiment 4 weeks old (R 29). The ash content in dry bone has been essentially reduced. Both the real and the percentage Ca and P content are considerably reduced.

In animals with an addition of cod-liver oil the water content has diminished, the ash content increased, and therefore a greater Ca and P content is found in dry as well as fresh bone, though everything is less than in animals on a normal diet with adequate salt content.

On a diet which is insufficient in A-vitamin and calcium a still more pronounced osteoporosis is produced which merely upon a hasty view seems to be somewhat veiled because the cartilage cells in the proliferous zone have ceased growing quicker and are therefore, as it were, congealed in a slightly differentiated stage, at which they remain, while resorptive osteoclastical processes cause a rarefaction and resorption of spongy trabeculae and compacta.

The very low ash content in the fat-free bone also shows that

in animals on plus A- $\div$ Ca-diet somewhat smaller bone tissue has been formed, so that besides the great water content in the bones there is also a great content of organic substance.

Investigations on the Effect of an A-vitamin-free and deficient Phosphorus Diet on Rats.

For this experiment were used 9 young rats from the same litter (32 days old).

The 7 rats were put on $\div$ A- $\div$ P-diet, while 2 animals had 3 per cent cod-liver oil added to the diet.

The animals ceased growing, perhaps a little sooner and more absolutely than animals on an ordinary $\div$ A-diet, but otherwise did not show any real differences from these. Death occurred after the animals had had the experimental diet for 6—8 weeks. The autopsy showed: keratomalacia, abscess at the root of the tongue, otitis media, cystopyelo-nephritis, abscesses of the salivary glands, fragile thin bones, often fractures of the ribs, emaciation.

The two animals (R 69 and R 70) receiving 3 per cent cod-liver oil added to the diet, show a steady constant rise of weight. The rise of the weight was slower and smaller than that of animals on normal diet. Autopsy of these animals showed otherwise absolutely normal conditions.

The histological examination showed that animals on $\div$ A- $\div$ P-diet suffered from osteoporosis, with low cartilage growth zone and arrested proliferation of the cartilage cells. Only few and thin compacta, very hyperaemic bone marrow, only in one case (R 66) was found slight osteoid in the bone, but in R 66, R 67, and R 71 was found distinct accumulation of non-differentiated cartilage cells in a high, rather regular layer in the epiphyseal line. The matrix was here uncalcified, the capsule preserved, and the cartilage cells somewhat retracted. The cells here are preserved, primitive cartilage cells, which, if the bone growth had been normal, would long since have been dissolved.

R 69 and R 70 which had 3 per cent cod-liver oil added to the diet, showed bones with processes of growth in a normal

way, but to a less extent than the normal bones, as the spongy trabeculae were thin and few; there was no osteoid tissue and no irregularity in the provisional calcification zone, no deficient calcification.

Schematic View of the Content of Water, ASH, Ca and P in Right Femur from Rats on $\div A$ - $\div P$ -Diet.

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur			
R 63	32	83	33	61	59.8	40.0	50.8	20.3	7.9	20.5	8.2	3.2	3.4
R 64	32	82	34	56	62.7	42.0	47.5	20.0	7.4	22.0	9.2	3.4	0.3
R 65	32	80	33	49	53.5	45.5	50.7	23.1	10.5	22.2	10.1	4.6	1.6
R 66	32	68	31	48	53.8	38.1	53.0	20.2	9.2	19.8	7.5	3.4	1.2
R 67	32	69	31	39	55.1	41.2	47.7	19.6	8.6	23.2	9.5	4.2	1.8
R 68	32	83	33	57	49.7	50.4	50.5	25.4	10.7	20.3	10.2	4.3	16.2
R 71	32	81	33	47	61.0	40.0	47.8	19.1	7.1	22.1	8.9	3.3	5.1
<i>$\div A$-$\div P$-diet plus cod-liver oil.</i>													
R 69	32	83	33	92	46.1	53.5	52.1	27.9	13.1	17.5	9.4	4.7	6.1
R 70	32	83	34	70	50.8	51.0	52.8	26.9	12.2	18.6	9.5	4.3	7.5
<i>Average of animals on $\div A$-$\div P$-diet</i>													
in all	7	32	78	33	51	56.5	42.4		8.7			3.7	
<i>$\div A$-$\div P$-diet plus cod-liver oil.</i>													
2	32	83	33	81	48.4	52.2			12.9			4.5	

The chemical bone examination showed increased water content, diminished ash content in dry fat-free bone, and reduced Ca and P content compared with animals on normal diet, and also compared with the animals on $\div A$ - $\div P$ -diet, but with 3 per cent cod-liver oil added to the diet. The P content was not, however, more reduced than that found in $\div A$ animals of the same age.

The development of the animals is thus considerably less inhibited on a $\div A$ - $\div P$ -diet than on a $\div A$ - $\div Ca$ -diet. It must

be remembered that the phosphorus content of the diet is not very low (0.2 p.c.), which is due to the abundance of phosphorus in the caseinogen. The bone deformities produced show certain rickety symptoms, but the predominant feature of the picture is cessation of growth and osteoporosis.

Investigations on the Content of Vitamin of various Butter Samples, judged of by the Influence on the Growth and the Bone Formation of young Rats.

Already at a very early stage of my experimental work and my endeavours to find experimental conditions so nearly resembling the conditions for the development of infantile rickets, and the effect of which on young rats might be said to resemble the clinical and pathologico-anatomical deformations of children so much, that the identity of the experimentally produced phenomena with the phenomena found in rickety children was made probable, my work received a very considerable impulse through the extensive series of investigations then started at the Hygienic Institute of the University.

These experiments, the object of which was to examine the content of growth-promoting vitamin in butter and the varying vitamin content in different butter samples, immediately provided part of the setting for my own experiments. They were carried out under the direction of Professor *Fridericia* by my colleague and friend Dr. *S. Gudjónsson* according to the method of *Zilva & Miura*. They were planned as feeding experiments by which the growth-promoting power of the butter samples could be estimated.

Upon autopsy of these experimental animals Dr. *Gudjónsson* observed that the bones from animals on $\div$ A-diet did not show anything abnormal macroscopically, while he observed that the bones in animals that had had certain daily additions of butter to the $\div$ A-diet macroscopically did show symptoms of rickets.

I soon realized the importance of this observation, and I am much indebted to Professor *Fridericia* and Dr. *Gudjónsson* for permission to utilize the great material of experimental animals

for special and more extensive investigations of rickets histologically as well as chemically.

As the experiments with the butter samples mentioned here only constitute a smaller part of the whole experimental plan, and as this has not been carried through yet, I shall here desist from a more exact report of the nature of the various butter samples and from estimating and discussing the importance of the feeding of cows, except with respect to the qualities of the milk and butter.

In the following I shall therefore merely refer to the butter as groups A, B, and D.

Examination of the Butter Group A.

Three samples of butter: A I, A II and A III were examined.

A I was of a more intensive yellow colour than A II, and A III was of the palest yellow colour.

This different colour of the three butter samples was very likely due to a varying content of pigments belonging to the carotinoid and xanthophyl group, and this has been connected with the content of liposoluble vitamin, it does not seem proved however (*H. Steenbock, Marianna T. Sell & Mary V. Buell* (246)).

The experiments were performed according to the method of *Zilva & Miura*. 30 days old the animals were put on the ordinary $\div$ -A-diet of the Institute and when a stand still of the weight was obtained in this way, the various animals had different amounts of the 3 butter samples added separately to the diet.

Some of the animals remained on $\div$ -A-diet, until the occurrence of death, some of them received 0.05 gm daily, 0.10 gm, 0.15 gm, 0.20 gm, 0.30 gm, 0.40 gm, 0.50 gm butter A daily.

As will be seen from the weight curves, an addition of 5 ctgm butter daily was not able to cause any growth. The animals remained stationary in weight from the third to the fifth week or even lost weight in the course of the 6th—7th week with spe-

cial feeding, and then died under symptoms of cachexy and under the same clinical symptoms as animals without any addition of butter. Neither can it be said that the animals were kept very much longer alive, as it is not at all unusual that animals on a $\div$ A-diet can live for 12—14 weeks on this diet.

The life of the animals is very much threatened by the suppurative inflammations that mostly occur; the longer the animal remains free from inflammations, (and if the more important organs are spared), the longer the animals will be able to live on a $\div$ A-diet, and even keep a fairly constant weight. The longest time I have seen such an animal live on a $\div$ A-diet is 19 weeks (134 days).

A daily addition of 10 ctgm of butter A is apparently able to increase the weight during the first 4 weeks after the feeding with this addition has commenced, but then the weight stops definitively, only to decrease at last. And death occurs under symptoms of cachexy and keratomalacia.

But an addition of 10 ctgm seems able to keep the animal somewhat longer and more safely alive, in contrast to $\div$ A-fed animals. Furthermore the autopsy of these animals showed that there was a greater or less amount of rickety fractures of costae and some thickening of the costo-chondral junction.

In this experiment butter A II seemed to yield a somewhat slighter impulse to growth than I and III, and the animals of group A II lived a shorter span of time than those from A I and A III.

A daily addition of 15 ctgm A was also able to produce some growth and to keep the animals at a certain increased weight, compared with $\div$ A-animals. Their weight did not rise further.

While some of the animals died from suppurative diseases, there were others which lived and were killed after having received the addition for 14 weeks.

In all these animals were found macroscopically distinct rickety deformities of costae. Some of these animals suffered from xerophthalmia.

In this experiment there was no difference in the influence

of the three butter samples, beyond the fact that the two animals on A II died spontaneously and earlier than the animals with the addition of A I and A III.

A daily addition of 20 ctgm A caused a distinct growth and some thriving of the animals, since there was not, even after 14 weeks of special feeding, any absolute cessation of growth of the animals fed on A I and A III, while the animals, which had received A II showed loss of weight. The animals on A I and A III had normal eyes, but divers abscesses and rickety symptoms. The animals on A II developed xerophthalmia and inflammations, as well as some rickety symptoms.

Thus in this case, too, A II proved less protective and less growth-promoting than A I and A III.

An addition of 30 ctgm A caused an even, slow and more constant rising of the weight. The weight curves of the animals kept on a level, without showing decisive differences for the three butter sorts. In this group none of the animals developed xerophthalmia, but the bones showed rickety fractures and thickening of the costal epiphyses.

The addition of 40 ctgm A per diem caused better and more rapid growth than 30 ctgm.

On autopsy the animals showed symptoms of healed abscess of the root of the tongue, still friable teeth and some fractures of costae, which, however, showed recovery with good calcification.

The experiment may perhaps be open to misinterpretation, since the two animals which got 40 ctgm A I per diem died spontaneously after 6—7 experimental weeks. After good growth in the beginning death supervened. Evidently the animals were not able to overcome the cystopyelitis, which had most likely occurred during the pure $\div$ -A-feeding.

50 ctgm A daily gave natural growth and thriving and seems to be sufficient in all respects.

Butter Group B.

In this group too, 3 samples of butter: B I, B II and B III were examined.

The control-animals on $\div$ -A-diet showed the ordinary well-known phenomena. There was some rise of the weight during the first 4 weeks, then cessation of weight, development of xerophthalmia, loss of weight. B 28 proved somewhat more resistant than the other animals and died 3 weeks later (lived for 108 days).

On autopsy were found the symptoms generally found in these animals. Histologically was found osteoporosis, only in B 28 and B 39 there were slight symptoms of rickets in costae, but no osteoid tissue.

Animals which, after they had ceased gaining in weight, had an addition of 25 mgm of butter B per diem, after this addition showed a slight rise in weight and a little longer life time than animals on $\div$ -A-diet, though not longer than we have already seen that it was in certain cases possible for such animals to live (B 28). After special feeding for 6 weeks, loss of weight occurred. They died spontaneously with the ordinary symptoms: friable, fractured teeth, abscess of the root of the tongue, otitis-media, fractures of the ribs, cystopyelo-nephritis. They all developed xerophthalmia, only in B 29, which received 25 mgm B II, it was not possible to prove any eye disease.

Histologically there was some osteoporosis in the bones, some inhibition of the growth but with preserved cartilage cell columns and uncalcified cartilage matrix in the provisional calcification zone. In some of the animals was found osteoid tissue, especially in the femur, in others not.

The addition of 5 ctgm B caused a slow but constant rise of weight and secured the life of the animals, only B 15 died spontaneously from cystopyelo-nephritis. These animals did not develop xerophthalmia, but as rule they showed numerous fractures of the ribs with hypertrophical soft callus and rosary. The

teeth were friable and a little thin, these animals did not show symptoms of suppurative inflammations (except B 13).

The weight curves were equal for the three butter sorts.

Histologically there was some osteoporosis of the bones and distinct rickets, as well in costae as in femur and tibia, and some osteoid tissue.

The addition of 10 ctgm B caused still better growth, even and constant, though not as much as an addition of 15 ctgm and upwards. The animals lived without hindrance. The three sorts of butter seemed to be of equal effect. Autopsy showed nothing abnormal, especially no xerophthalmia or inflammations. The teeth were still a little friable and thin.

Histologically some osteoporosis and distinct rickets with some osteoid tissue were still seen.

The addition of 15 ctgm B caused a still more rapid and even growth.

It can hardly be supposed that the somewhat scattered course of the curves is to be explained as a consequence of a different growth-promoting power of the three butter sorts, since B 41 and B 50 belonged to the same litter, while B 51, B 52, B 54 and B 56 all belonged to another litter of rats. On post mortem view of these animals a smaller or greater amount of rather compact fractures of the ribs was found, but otherwise macroscopically hardly anything certainly abnormal. On the other hand microscopically slight osteoporosis, and pronounced rickets with some osteoid tissue were found.

The addition of 20 ctgm B caused a rapid and constant rise of weight.

The autopsy presented some healed fractures of ribs, otherwise macroscopically hardly anything abnormal.

Histologically B 22, B 25 and B 23 and B 26 still showed slight osteoporosis, and pronounced rickets with some osteoid tissue, while B 30 and B 35 perhaps still showed somewhat few and thin spongy trabeculae, but altogether presented a nearly normal picture of the skeleton.

From this experiment also, in which it is seen that B 30 and B 35 grew more rapidly and better than the four other animals, and even showed a nearly normal bone picture, we dare not suppose that this is due to more strongly acting qualities of the butter B III, since B 35 did not belong to the same litter of rats as the other 4 animals.

The addition of 30 ctgm B seems to yield the same conditions as a normal diet, though 40 ctgm B causes still better conditions, which may especially be seen by the fact that the animals recover more rapidly and more surely, and show a quicker rise of weight after they have been exposed to the injurious effects of the $\div$ -A-diet.

Histologically the two groups show normal bones.

Schematical View of the Content of Water, ASH, Ca and P in the Right Femur from Rats with a varying Addition of Butter in the Diet.

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur			
butter 0.025													
B 33	32	106	34	81	54.3	42.7	50.8	21.8	9.4	19.2	8.2	3.6	0.5 I
B 37	32	103	34	56	56.9	42.1	48.1	20.3	8.7	19.8	8.3	3.6	0.2 »
B 29	32	111	34	69	46.3	42.9	49.0	21.0	9.1	20.4	8.7	3.7	1.2 II
B 36	32	94	34	73	53.4	46.5	47.8	22.2	9.6	18.4	8.5	3.7	2.9 »
B 31	32	108	33	64	59.0	46.8	50.6	23.7	9.5	20.6	9.6	3.8	2.3 III
B 34	32	101	34	78	57.1	45.7	49.4	22.6	9.8	18.3	8.4	3.6	0.1 »
Average													
	6	32	104	34	70	54.5	44.4		9.3		3.6		
butter 0.05													
B 10	34	141	38	105	52.3	46.1	51.9	23.9	10.5	18.1	8.3	3.7	7.4 I
B 13	34	122	46	81	53.3	44.5	50.5	22.9	10.0	18.0	8.4	3.7	0.5 »
B 11	34	141	35	88	50.8	44.3	52.1	23.1	9.9	22.2	9.8	4.2	12.3 II
B 14	34	141	31	85	52.6	48.1	51.5	24.8	10.9	20.2	9.7	4.3	6.6 »
B 18	34	141	36	110	47.9	49.2	50.0	24.6	11.0	17.3	8.5	3.8	10.1 »
B 9	34	141	38	102	47.6	49.5	51.3	25.4	12.4	17.5	8.6	4.2	7.0 III
Average													
	6	34	138	37	95	50.7	46.9		10.7		3.9		

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat	
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur				
butter 0.1														
B 12	34	141	37	95	48.7	49.5	50.2	24.8	11.7	18.1	8.9	4.2	8.4	I
B 15	34	141	37	103	50.0	48.4	50.5	24.4	11.3	18.0	8.7	4.0	7.6	»
B 16	34	141	34	105	49.5	50.4	49.3	24.8	11.7	20.3	10.2	4.8	6.8	II
B 19	34	141	36	106	46.7	50.6	50.0	25.3	11.3	18.0	9.1	4.0	16.1	»
B 20	32	139	45	128	44.4	53.9	49.0	26.4	13.4	18.4	9.9	5.0	8.5	III
B 21	32	139	36	90	44.4	51.2	49.2	26.2	12.5	18.0	9.6	4.6	13.9	»
average														
6	34	141	37	104	47.2	51.0			12.6		4.4			
butter 0.15														
B 41	32	115	33	100	49.7	47.2	51.6	24.4	11.8	18.6	8.7	4.2	3.7	I
B 50	32	115	35	98	42.1	55.3	52.3	29.0	16.6	18.0	10.0	5.7	1.0	»
B 51	32	111	34	106	46.8	49.5	51.0	25.2	12.2	19.4	9.6	4.6	9.3	II
B 56	32	111	40	135	49.0	48.6	51.0	24.8	11.1	19.7	9.7	4.3	12.1	»
B 52	32	111	40	133	47.5	48.0	50.1	24.4	11.4	19.4	9.4	9.3	4.3	III
B 54	32	111	42	145	46.2	49.7	50.9	25.3	11.9	17.8	8.8	4.1	12.3	»
average														
6	32	112	37	119	46.8	49.7			12.5		4.5			
butter 0.2														
B 22	32	139	41	124	46.9	51.3	51.4	26.3	12.8	16.5	8.4	4.1	8.1	I
B 25	32	139	40	104	46.7	48.3	50.0	24.1	10.6	18.2	8.8	3.8	17.4	»
B 23	32	122	37	122	43.2	54.5	50.9	27.8	14.1	17.7	9.7	4.9	10.3	II
B 26	32	139	43	107	45.2	51.5	50.5	26.0	12.5	17.9	9.2	4.4	11.9	»
B 30	32	129	36	156	45.3	53.4	51.3	27.4	13.6	17.6	9.4	4.6	9.4	III
B 35	32	129	32	126	42.3	56.6	51.3	28.8	15.8	16.2	9.1	5.0	4.5	»
average														
6	32	132	38	123	44.9	52.6			13.2		4.4			
butter 0.3														
B 44	32	115	35	119	44.0	53.5	50.6	27.1	14.4	19.4	10.3	5.5	5.0	I
died														
B 47	32	66	31	40	62.4	34.7	52.2	18.1	6.7	19.2	6.6	2.5	0.5	»
days old														
B 53	32	111	39	141	44.8	51.2	49.5	25.4	12.4	18.1	9.2	4.9	11.2	I
B 49	32	115	33	128	49.8	47.2	48.3	22.8	11.0	18.9	8.9	4.3	3.9	II
B 40	32	115	33	131	44.6	52.7	51.1	27.4	13.9	17.7	9.3	4.7	5.0	III
B 43	32	115	30	127	48.1	51.5	51.7	26.6	13.1	18.1	9.3	4.6	5.1	»
B 45	32	115	33	140	46.1	52.0	50.3	26.1	13.4	18.0	9.3	4.8	4.6	»
average														
6	32	114	34	131	46.2	51.5			13.0		4.8			

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh fe.mur	ash of dry fat free femur	of fresh femur	of fresh femur		
butter 0.4													
B 57	32	111	37	147	47.1	51.2	50.8	26.0	12.2	18.1	9.3	4.3	11.1 I
B 59	32	111	36	131	52.3	49.6	50.8	25.2	11.4	19.1	9.5	4.3	3.0 »
B 55	32	111	36	131	43.4	56.3	52.2	29.4	15.6	17.9	10.0	5.3	6.4 II
B 60	32	111	37	139	44.2	55.2	52.0	28.7	14.8	17.1	9.8	5.0	7.3 »
B 61	30	122	27	139	43.5	58.4	52.9	30.7	16.1	17.4	10.2	5.3	7.7 III
B 62	30	122	26	125	41.8	59.5	52.8	31.4	17.0	16.6	9.9	5.3	6.7 »
average													
6	32	114	33	135	45.5	55.0			14.5			4.9	

From the table of the chemical results of the examinations of these bones it will be seen that the increasing addition of butter causes a constant decrease of the water content of the bones and a constant increase of the ash content, as well as the fat content. In judging of these figures the age of the animals must be taken into account.

In comparing the percentage content of water in femur with detailed tables of normal bones of rats mentioned by *R. M. Strong* (253) (cit. *Henry H. Donaldson* (41)) and *Frederik S. Hammett* (80), it will be seen that the water content is considerably higher than would correspond to the amounts found by *Strong*. Only the animals receiving 0.4 gm butter per diem have a normal low water content.

That the table shows a very oscillating fat content in the bones must no doubt be explained by the fact that amounts of fat that cannot be verified have partly evaporated, partly melted away during the stay of the bone in the kiln, before the extraction with petroleum-ether was made.

As two animals hardly behave alike in all respects, the animals being from different litters, and as furthermore the results may be influenced within certain limits by investigation errors, it is only permissible if great caution is exercised to draw any conclusions as to whether one butter sort has shown a higher ability to deposit calcium and phosphorus in the bones than

another butter sort. While B I and B II seem to be on a level, in B III there is found a distinct tendency to cause greater deposition of Ca and P in the bones.

Butter Group D.

Also in this group I shall not enter more closely upon the subject of the feeding of the cows. I shall only mention that in this case too, three sorts of butter have been examined, viz. D I, D II and D III.

First I shall briefly mention the control-animals of these experiments, as two of the animals proved extraordinarily resistant to the injurious influence of the $\div$ A-diet. The first stood this diet for 92 days, and lived to the age of 126 days; the other, D 31, was on $\div$ A-diet for 134 days, and lived to the age of 161 days. All three control-animals died at last under the same clinical picture: emaciatio, xerophthalmia to anophthalmia, furthermore suppurative inflammations.

An addition of 25 mgm D per diem to young rats, which, by feeding with $\div$ A-diet for 5 weeks had ceased growing and were already beginning to show loss of weight, caused the loss of weight to stop, there might even be a slight temporary rise, but after the course of 5—6 weeks the animals lost in weight and death occurred, hardly much later than it is often the case with animals on a pure $\div$ A-diet. The animals died under the usual symptoms of A-avitaminosis: xerophthalmia, emaciatio, and suppurative inflammations, but furthermore they all presented a macroscopic picture of rickets in costae.

Histologically some osteoporosis in the bones was found, besides slight retardation of the growth with preserved cartilage cell columns and isles surrounded by an uncalcified cartilage matrix, as well as some osteoid tissue.

That butter D I should be able to keep the animals alive longer than the two other sorts of butter, cannot be inferred from the curves, since, as already mentioned, animals on a $\div$ A-diet may show similar individual differences.

An addition of 5 ctgm caused some, but slow and uncertain, rise of weight and after about 5—7 weeks of special feeding, cessation of gain, and the three following weeks loss of weight.

While during the preparatory 6 weeks of $\div$ A-feeding, gain of weight ceased and xerosis conjunctivae was developed, it was observed that these symptoms again diminished during the addition of butter, but in some of the animals occurred once more during the renewed and definitive cessation of gain (D 12, D 4). In D 10, D 11, D 14, and D 1 there were no distinct symptoms of xerosis conjunctivae, even if the borders of the eye-lids were still hairless, and there was some enophthalmus in some of the animals. No abscesses were found but macroscopically pronounced rickety deformations.

Histologically all these animals besides some osteoporosis showed very considerable rickety deformations with enormous derangement in the provisional calcification zone, this being considerably enlarged, with long rows and lengthened isles of non-differentiated cartilage cells in the uncalcified cartilage matrix, these extended far into the marrow cavity; in between these isles there were wider and narrower marrow cavities and blood vessels, further, great amounts of osteoid tissue.

An addition of 10 ctgm D also caused a slow increase in weight, which after the course of some weeks tended towards cessation. The animals were still alive, however, and on autopsy, after 10—11 weeks of special feeding, no symptoms of xerosis conjunctivae or suppurative inflammations were found. Macroscopically, violent rickety symptoms were found: fractures of costae, rosary, deformities of sternum, thorax and columna, clumsy bones of the limbs.

Histologically osteoporosis was found, less prominent than in the preceding group, but otherwise the picture was remarkably instructive for rickets.

The three groups of butter hardly showed any difference.

An addition of 15 ctgm D gave a better, though not normal rise in weight. The increase of weight showed no certain tenden-

cy towards cessation in spite of 11 weeks' special feeding.

Upon autopsy found fractures of costae and more or less pronounced rickety deformations of thorax and the vertebral column, no suppurative inflammations and no symptoms of eye disease.

Histologically found pronounced rickets.

An addition of 20 ctgm D caused an even, but slow, increase of weight.

Upon autopsy found rickety deformations. D 36 suffered from abscess of the root of the tongue.

While D 23 in the main presented a normal histological picture of the bones, the bones of the other animals still showed rickets, but hardly any osteoporosis.

An addition of 30 ctgm D caused somewhat more advantageous conditions. D 35 and D 37 were stationary in weight for some weeks. Upon autopsy no cause could be proved. These two animals showed some slight symptoms of rickets, while the bones from the other animals of this group must be called normal.

An addition of 40 ctgm D gave normal conditions for rise of weight and after feeding with this addition the osseous system showed a normal histological picture.

As mentioned before D 35 and D 37 of the preceding experimental group showed cessation of the developed rise in weight. This occurred, when the animals were 110—120 days of age. A similar tendency was seen in this group as regards D 44 and D 43, in this case even in spite of an addition of 40 ctgm. The reason for this seems to be that these animals were kept 1—2 weeks longer on a $\div$ A-diet before an addition took place, as against the other animals, which got the addition somewhat earlier.

It may also seem surprising that the animals in the group with the addition of 20 ctgm per diem did not grow faster, though this addition did make them grow a little and neither xerophthalmia nor inflammations supervened, in spite of 14 weeks' special feeding. Also these animals were kept 6 weeks on $\div$ A-diet, while in many of the preceding experiments the addition was made after 4—5 weeks of feeding with $\div$ A-diet.

Therefore, there is reason to suppose that the cessation of growth which the $\div$ A-diet causes in the epiphyseal cartilage cells is considerably more serious and definitive the longer the animals have been fed with this diet, so that the epiphyseal line calcifies and ossifies more rapidly and thus prevents further longitudinal growth of the bones.

This retardation of further growth in spite of adequate experimental conditions cannot be fully explained by supposing that the animals have at this age (110—120 days) attained physiological closing of the epiphyseal lines on account of adult age. From the experiment made on normal rats by *Dawson* it thus appears that the proximal epiphyseal line of ulna and the distal epiphyseal line of femur and proximal epiphyseal line of tibia, fibula and humerus do not disappear until the animal is 25—35—37 months old.

Schematic View of the Content of Water, ASH, Ca and P in Right Femur of Rats with different Additions of Butter in the Diet.
Butter D.

No of rat	Age in days at the		weight at the		H_2O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent				per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur	fat free femur	of fresh femur		
D 8	34	126	31	81	56.6	46.6	53.0	24.6	10.6	17.3	8.0	3.4	0.5	
D 27	33	75	32	50	58.9	47.2	54.1	25.5	9.3	26.5	12.5	4.5	11.4	
D 31	31	165	37	68	55.4	42.5	51.6	21.9	9.7	22.9	9.7	4.3	0.6	
													butter 0.025	
D 24	33	120	37	67	61.1	44.4	52.1	23.1	8.9	19.5	8.7	3.3	0.9	I
D 28	33	131	40	52	59.4	44.7	50.6	22.7	8.8	21.2	9.5	3.7	4.2	»
D 26	33	105	39	75	57.7	46.8	44.3	20.7	8.5	21.8	10.2	4.2	2.8	II
D 29	33	101	37	76	59.5	47.4	50.7	24.1	9.3	21.0	10.0	3.8	4.6	»
D 25	33	90	35	63	60.8	38.1	52.2	19.9	7.8	26.5	10.1	3.9	0.2	III
D 30	33	113	40	62	57.8	45.0	52.1	23.5	9.5	23.5	10.6	4.2	4.0	»
average														
	6	33	110	38	66	59.4	44.4		8.8		3.8			
													butter 0.05	
D 10	32	158	44	109	48.3	47.6	56.4	26.9	12.9	17.8	8.5	4.0	9.0	I
D 12	32	127	48	71	52.7	40.2	51.2	20.6	8.7	20.8	8.3	3.5	10.4	»
D 11	32	158	45	118	50.6	44.6	53.1	23.7	10.2	24.9	11.3	4.9	8.7	II

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur	of fresh femur	of fresh femur	
D 14	32	158	45	102	48.2	48.5	50.3	20.5	11.5	17.4	8.4	4.1	6.0 »
D 1	34	162	30	92	45.3	51.6	52.4	27.1	13.7	19.2	9.9	5.0	7.4 III
D 4	34	162	36	116	46.5	46.5	57.2	26.5	13.1	16.4	7.6	3.7	7.5 »
average	6	33	154	41	101	48.6	46.5		11.6		4.2		
												butter	0.1
D 2	34	162	33	111	40.2	53.8	53.3	28.7	15.5	18.6	10.0	5.4	9.6 I
D 6	34	162	35	115	53.7	44.7	51.6	23.1	9.7	23.8	10.6	4.4	9.4 »
D 3	34	162	34	108	48.0	48.4	53.6	26.5	12.7	19.7	9.5	4.6	7.0 II
D 5	34	162	35	115	51.2	46.1	50.6	23.4	10.5	19.8	9.1	4.1	7.8 »
D 7	34	162	36	95	50.0	43.5	52.3	22.7	10.0	19.4	8.7	3.7	11.5 III
D 9	34	162	34	116	45.2	51.2	51.8	26.5	13.3	19.3	9.9	4.0	8.2 »
average	6	34	162	34	110	48.0	47.9		11.9		4.5		
												butter	0.15
D 15	32	157	35	117	46.9	47.3	52.1	24.6	11.3	23.2	10.9	5.0	13.4 I
D 19	32	157	36	108	47.3	48.7	52.1	25.4	11.8	20.8	10.1	4.7	11.8 »
D 16	32	157	35	109	48.3	44.1	50.8	22.4	10.4	19.8	8.4	4.0	9.9 II
D 21	32	157	31	101	44.0	51.0	52.3	26.7	12.0	20.1	10.2	5.3	6.6 »
D 17	32	157	35	114	50.2	46.4	51.0	23.7	10.9	20.3	9.4	4.3	7.7 III
D 18	32	157	35	127	47.4	48.5	53.2	25.8	11.6	19.1	9.3	4.1	14.3 »
average	6	32	157	34	112	47.3	47.0		11.6		4.5		
												butter	0.2
D 22	32	157	34	135	46.8	47.9	53.9	35.8	11.5	17.1	8.2	3.6	16.1 I
D 23	32	157	34	127	41.8	53.2	51.9	27.6	14.4	18.3	9.7	5.1	10.3 »
D 32	32	173	37	113	47.2	52.2	52.6	27.4	13.7	18.2	9.5	4.7	5.0 II
D 34	32	173	39	94	44.8	50.9	51.6	26.3	11.0	20.4	10.4	4.3	23.7 »
D 33	32	173	39	135	47.5	49.6	54.7	27.2	12.8	19.0	9.4	4.4	9.7 III
D 36	32	173	36	103	46.1	55.9	51.5	28.8	14.5	19.2	10.7	5.4	6.2 »
average	6	32	167	36	118	45.7	51.6		12.9		4.6		
												butter	0.3
D 35	32	173	39	107	45.3	56.4	51.5	29.8	15.1	18.2	10.3	5.3	4.8 I
D 37	32	173	37	122	46.0	52.4	50.7	26.5	11.7	19.4	10.2	4.5	18.1 »
D 38	34	167	30	133	43.6	57.5	53.0	30.5	16.7	18.8	9.7	5.3	2.5 II
D 39	34	167	30	124	40.8	59.3	52.8	31.3	17.4	16.3	9.6	5.3	5.9 »
D 40	34	167	31	143	41.7	55.4	53.3	29.5	15.6	16.2	9.0	4.7	9.1 III

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur	
D 41	34	167	36	145	46.3	53.2	49.5	26.3	13.0	16.3	8.7	4.3	8.0 »
D 46	31	154	31	170	44.1	53.3	53.9	28.7	13.7	17.3	9.2	4.4	14.6 »
average	7	33	167	33	157	43.9	55.3		14.7		4.8		
												butter	0.4
D 43	34	167	36	142	43.7	54.0	48.8	26.4	13.3	16.3	8.8	4.4	10.1 I
D 49	31	154	30	183	43.8	53.0	52.1	27.6	13.5	16.5	8.7	4.2	9.6 »
D 44	34	167	27	115	46.7	51.2	53.2	27.2	12.9	17.6	9.0	4.3	10.7 II
D 51	31	154	32	175	47.6	51.7	49.8	25.7	12.3	16.4	8.4	4.0	8.7 »
D 47	31	154	31	178	43.3	52.3	49.5	25.9	12.7	17.0	8.9	4.3	10.0 III
D 48	31	154	29	133	38.5	57.6	53.9	31.0	17.7	17.7	10.2	5.6	10.0 »
D 50	31	154	30	168	44.9	53.5	53.5	28.6	14.0	16.5	8.8	4.3	11.1 »
average	7	32	157	30	156	44.0	53.3		13.7		4.4		

The chemical examination of the bones of these animals showed the same conditions as previously mentioned. With the increasing addition of butter is found a diminishing content of water in the bones and an increasing ash content, especially shown by the content of Ca and P.

In judging of the figures the age of the animals has to be considered, as the ash content increases with the age. These conditions have been thoroughly studied by *Henry H. Donaldson* (41) and *Frederick S. Hammett* (80). *Hammett* has determined the content of ash, organic substance and water in normal bones from rats in the growing period, as well as the composition of the ash. Such animals, however, that receive insufficient amounts of butter, and in consequence get emaciated, will be able to show a decreasing percentage content of ash, calculated from dry, fat-free bone, if they have been living for a longer period, — become more intensely emaciated and got more osteoporotic bones, — than animals with an equal addition, but which died at an earlier date (compare D 8, D 27, D 31, D 25 and D 30).

The schematic view gives no proof that the calcium content of bones from animals fed with one of the butter sorts, is more intense than in animals, which have had another butter sort.

Experiments on Feeding by the Addition of Butter to a $\div$ A-diet without Preceding Cessation of Growth.

Butter B I.

These experiments too were tried on young rats, which about 30 days old commenced on our special $\div$ A-diet, but directly at the beginning of the experiment were given different additions of butter.

As will be seen from the curves of these experimental animals, the rise of weight during the first three weeks was very regular and fairly equal, whether the animals got $\div$ A-diet or this diet with differing additions of butter. During the 4th week the differences appeared. The animals with the big additions of butter now increased more rapidly in weight, while the weight curves of the animals getting 0.025 gm and less showed cessation of the gain in weight and at last cessation of growth. Animals with 0.1 gm daily are just over the limit for preserved growth. That C 3 and C 4 in the last week before death occurred showed a stationary weight, is due to an intercurrent disease: enteritis. It will also be seen from the B-experiments that 0.1 gm daily of the 3 butter sorts secures growth.

If the animals get additions of butter so small that the rise in weight is not secured, it will be seen that these animals having had the addition from the beginning of the experiment, at no period reach an essentially higher weight than the animals which have first been stopped in growth. But it appears as if the first mentioned animals can be kept alive for a longer period (C 11 and C 10). This is very likely due to a later occurrence of the suppurative inflammations.

The animals showing definitive cessation of growth get xerophthalmia, and the occurrence of this, is seen to coincide with the occurrence of cessation of growth.

Animals with an addition of 0.1 gm and upwards do not get xerophthalmia and these animals also show preserved growth.

Autopsy of animals on $\div$ A-diet and of animals with an addition of 0.025 and 0.05 gm butter showed xerophthalmia to anophthalmia, suppurative inflammations in the different organs (processus mastoideus, root of the tongue, salivary glands, urinary organs etc.) as well as emaciation. Further, these animals macroscopically showed rickets, rosary, costae-fractures and deformations of thorax, the latter symptoms, however, were not found with pure $\div$ A-feeding.

Animals getting 0.2 and 0.3 gm addition upon autopsy showed nothing abnormal.

Histologically was found pure osteoporosis with $\div$ A-diet. By the addition of 0.025 (C 8, C 11) considerable osteoporosis was found with more or less pronounced derangement and enlargement of the provisional calcification zone. This zone was incompletely calcified and the cartilage cells had remained in a non-differentiated stage. Furthermore some osteoid tissue was found.

In the animals getting 0.05 gm daily osteoporosis and rickets were found, as in the preceding group.

The addition of 0.1 gm still caused some osteoporosis and distinctly pronounced rickets with some osteoid tissue.

The addition of 0.2 and 0.3 gm caused normal development of the bones.

The chemical bone examination showed conditions similar to those mentioned before. As the final ages of the animals in this group of the experiments differed so much, I shall not try to compare the separate figures, only draw attention to the great differences of the water content in the bones of the animals of the same age, but fed with different additions of butter: C 11 (223 days old), 54.1 per cent H_2O , C 10 (230 days old) 47.1 per cent H_2O , against C 12 and C 13 with respectively 36.1 and 31.6 per cent H_2O . C 3 and C 4 (114 days old) with respectively 54 and 57 per cent H_2O against C 1 and C 2 (121 days old) with about 45 per cent H_2O .

Schematic View of the Content of Water, ASH, Ca and P in Right Femur from Rats with various Additions of Butter in the Diet, without preceding Cessation of Growth.

No. of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent		P per cent		per cent fat		
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur			
without addition.													
C 7	30	65	26	45	58.9	37.7	44.9	16.9	6.8	24.9	9.4	3.7	2.2
Addition of 0.025 from begin. of experiment.													
C 8	33	151	36	96	54.3	46.7	51.5	24.0	10.9	19.9	9.3	4.2	0.2
C 11	33	223	30	84	54.1	50.5	52.9	26.7	12.2	17.1	8.6	3.9	4.4
							—»—	0.05	—»—	—»—			
C 6	30	86	28	65	60.4	41.8	48.5	20.3	8.0	23.7	9.9	3.9	0.4
C 9	33	150	32	73	59.2	46.6	52.6	24.5	9.9	17.0	7.9	3.2	0.2
C 10	33	230	30	109	47.1	51.1	50.9	26.2	13.5	17.8	9.1	4.7	2.6
							—»—	0.1	—»—	—»—			
C 3	30	114	26	100	53.7	47.8	52.0	24.9	11.4	20.6	9.8	4.5	0.3
C 4	30	114	28	89	56.7	44.4	52.1	23.1	9.9	22.4	9.9	4.2	0.8
							—»—	0.2	—»—	—»—			
C 1	30	121	26	142	45.4	56.6	53.0	30.0	15.4	17.4	9.1	5.0	6.1
C 2	30	121	29	140	44.5	56.6	52.7	29.8	14.9	18.3	10.4	5.2	9.8
							—»—	0.3	—»—	—»—			
C 12	33	230	30	192	36.1	58.8	50.7	29.8	17.4	17.0	10.0	5.8	8.3
C 13	33	230	29	137	31.6	63.4	52.0	33.0	21.7	16.8	10.7	7.0	3.8
Butter Diet without addition													
C 24	35	88	27	78	55.1	50.6	50.3	25.5	10.8	18.6	9.4	4.0	3.6
C 28	35	103	28	74	53.6	46.0	52.2	24.0	10.6	19.0	8.2	3.8	0.4
Addition of 0.025 from beg. of experiment.													
C 15	32	125	39	70	60.7	42.6	49.9	21.2	8.3	21.2	9.0	3.5	0.4
C 16	32	97	36	78	54.6	43.9	50.0	21.9	9.6	23.1	10.1	4.4	3.6
							—»—	0.05	—»—	—»—			
C 14	32	154	34	91	59.8	50.6	53.4	27.0	13.0	18.4	9.3	4.5	4.3
C 17	32	69	41	81	57.2	45.7	52.4	23.9	10.1	19.8	9.0	3.8	0.8
							—»—	0.1	—»—	—»—			
C 18	32	154	40	121	48.8	52.1	57.3	29.9	14.3	16.9	8.0	4.2	6.7
C 19	32	154	45	123	51.0	48.9	49.0	23.9	10.7	17.1	8.3	3.7	8.6
							—»—	0.2	—»—	—»—			
C 20	32	154	43	133	46.8	54.4	52.6	28.6	13.9	16.0	8.7	4.2	8.7
C 21	35	80	29	95	53.7	50.0	53.7	26.7	12.3	17.7	8.8	4.0	0.6
							—»—	0.3	—»—	—»—			
C 22	35	137	29	143	39.4	55.6	52.2	29.0	14.7	17.8	9.9	5.0	16.5
C 25	35	137	31	116	43.6	56.0	52.2	29.2	15.4	16.8	9.4	4.9	6.6

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat	
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur	of fresh femur			
—»— 0.4 —»— —»—														
C 23	35	137	21	123	44.2	56.1	52.5	29.5	15.7	17.3	9.7	5.1	4.7	
C 27	35	137	29	150	46.0	54.7	52.5	28.7	14.3	16.4	9.0	4.5	7.4	
Control-experiment with butter A I, stored for one year, on rats after preceding cessation of growth.														
														gm
D' 52	31	89	31	58	64.9	43.5	51.4	22.4	7.8	24.0	10.4	3.6	0.4	0.1
D' 53	31	86	36	64	59.0	42.9	51.6	22.2	8.6	21.0	9.0	3.5	5.2	->-
D' 54	31	147	36	113	48.7	50.9	51.2	26.0	11.7	16.6	8.4	3.8	12.1	0.2
D, 55	31	147	36	124	41.3	57.2	53.0	30.3	16.3	17.0	9.7	5.2	8.2	->-
D' 56	31	75	36	45	62.1	42.1	50.4	21.2	8.0	20.8	8.8	3.3	0.9÷A	

Butter D I.

These experiments in all respects agree very nearly with the just mentioned experiments with butter B I. Here, too, the limit for the dose of butter able to secure the growth lies just under 0.1 gm daily, as the animals with 0.025 gm and 0.05 gm added to the diet got xerophthalmia, emaciation and purulent inflammations, macroscopically some rickets was seen.

Animals with 0.1 gm preserved growth, though slight, and did not develop xerophthalmia or purulent inflammations, but a more pronounced macroscopic rachitis.

Animals with 0.2 gm still showed rickets, which was not the case with butter B I (C 21 died from pneumonia).

Not until the animals received 0.3 and 0.4 gm daily did they prove normal in all respects.

Histologically considerable osteoporosis without symptoms of rickets was found in C 24, C 26, C 28 (÷A-diet).

C 15 and C 16 got 0.025 gm. Considerable osteoporosis was found here. In C 15 were found slight symptoms of rickets in costae with preserved cartilage cell columns and small amounts of osteoid tissue, while C 16 only showed osteoporosis.

C 14 and C 17 got 0.05 gm. C 14 showed some osteoporosis

and distinctly pronounced rickets, C 17, on the contrary, only osteoporosis and only slight symptoms of retardation of growth. C 17 died suddenly after having been 4 weeks in experiment. No cessation of growth had yet appeared, and no xerosis corneae.

C 18 and C 19 had 0.1 gm. Here the findings were severe rickets, less osteoporosis. C 20 had 0.2 gm and showed some rickets, while C 21 which also had 0.2 gm in the main showed a normal bone picture, when after 5 weeks' experiment it died from pneumonia.

C 22 and C 25 had 0.3, C 23 and C 27 0.4 gm daily addition. In those 4 animals the histologic bone picture was absolutely normal.

The chemical examination showed conditions similar to those mentioned under the preceding experiment.

As a last experiment in this series investigating the content of growth-promoting A-vitamin in different butter samples, I shall finally mention a little control experiment of one of the butter A samples kept for one year in a refrigerator.

The experiment was performed with 5 rats from the same litter. They were put on a $\div$ A-diet until the growth ceased. This occurred in the usual way after the course of 5 weeks. D' 56 got no addition. D' 52 and D' 53 had 0.1 gm A I daily, they died after the course of 3 weeks from xerophthalmia, and purulent inflammations and emaciatio. Histologically was found osteoporosis, without symptoms of rickets in these 3 animals.

D' 54 and D' 55 had an addition of 0.2 gm daily and showed a normal and good rise of weight.

Histologically a normal bone picture was found in these animals. The chemical examination corresponded with these findings.

By administering different samples of butter as an addition to an A-vitamin-free diet, which in all other respects was adequate, we can deduce the following results from the effects on the organism of the experimental rats.:

- 1) Judging from the weight curves of the experimental ani-

mals there is a marked difference between the content of growth-promoting A-vitamin in the different groups of butter.

2) The rise in the weight of the young rats fed with the same butter sample is proportional to the size of the given addition of butter, that is to say, if this has such a size that the rise of the weight is secured.

3) The different sorts of butter A have a varying content of A-vitamin, judged by the ability of the butter to promote growth, to keep the animals alive, and to cause deposition of minerals in the bones.

4) The water content of the bones decreases and the ash content (especially Ca and P) rises in fairly direct ratio with the size of the butter addition given.

5) Animals on a pure $\div$ A-diet get xerophthalmia as soon as they cease to gain in weight. But animals receiving such additions of butter which prove insufficient to secure the growth, also get xerophthalmia, when cessation of growth supervenes.

6) Animals with a secured rise of weight never get xerophthalmia, and if this has already appeared, when a butter addition sufficient to develop growth is given to the animal, the xerophthalmia will be healed.

7) Animals with arrested growth caused by deficient A-vitamin get suppurative diseases in the most various organs: keratomalacia, panophthalmia, otitis media, mastoiditis, abscess of the root of the tongue, inflammation of the salivary glands, pneumonia, cystitis, pyelitis, pyelo-nephritis, prostatitis, inflammation of epididymis and vesiculae seminales. While animals with secured growth escape these affections, or if they have already occurred, the addition of butter causes healing of the diseases.

8) All animals with the above-mentioned cessation of growth show osteoporosis in the histological bone picture, as well as some slight symptoms of rickets.

9) All animals with an addition of butter larger than that just required for the growth, but less than that required for normal growth and a normal rise of weight, show undoubted rickets, rachitis florida.

10) Animals with so large an addition that the rise in weight is normal, show a normal bone picture.

11) These facts are further confirmed by the chemical bone analyses.

12) Healing of experimental rickets takes place with each of the examined butter sorts and is only dependent on the size of the butter addition. Hence it is a question of dosage.

13) The cause of the different content of vitamin in the butter samples must be sought in the different fodder given to the three groups of cows.

14) There is a distinct, broad range of variation in the size of the additions of the various butter samples required respectively for the prevention of xerophthalmia, for some growth, but with the supervision of rickets, and for good growth with normal development of the bones.

The Influence of an Adequate Diet to young Rats arrested in Growth on a $\div$ -A-diet.

To investigate how animals arrested in growth on an A-vitamin-free diet can begin to grow again, how soon the mineral salts are deposited in the bones, and whether the growth in these, previously osteoporotic, bones takes place normally from the moment the animal receives the adequate diet, the following experiments were carried out.

9 young rats of the same litter when 35 days old were put on our $\div$ -A-diet. When growth ceased, after 4 weeks all the animals were (65 days old) put on our normal diet (which contains 15 per cent of butter fat). The weight of the animals rose immediately at the beginning of the feeding, as may be seen from the curves. The rise in weight was continual and rather high. The animals were killed after 1, 2, 3, 4 and 5 weeks.

R 47 was killed after having had N-diet for 4 days. Histologically its bones showed some osteoporosis, but distinctly commencing proliferation of the cartilage, commencing growth, no irregular growth, no enlargement of the calcification zone. R 48

and R 49 also showed a normal bone picture histologically, perhaps the spongy trabeculae were as yet somewhat sparse. R 50—R 55 inclusive also showed normal conditions.

The chemical bone analyses showed a rapid and constantly increasing deposition of minerals in the bones.

The Influence of a subminimal daily Addition of Butter to young Rats arrested in Growth on a $\div$ A-diet.

7 young rats of the same litter were (30 days old) put on a $\div$ A-diet. After 6 weeks, when the animals were 70 days old, a daily addition of 0.1 gm butter A was given, administered in the form of pills (constituents: purified starch). The animals were killed or died spontaneously, a few or several days after the special feeding had begun.

The animals had fallen off rather during the 6 weeks of A-free diet; in spite of additions of butter, they at once lost weight rather quickly and died under the usual symptoms of A-avitaminosis.

The histological examination showed osteoporosis in all the animals, without any symptoms of rickets.

An experiment similar to this was performed with 10 young rats of the same litter, but when 45 days old, immediately from the beginning of the experiment these were given a daily addition of 0.1 gm butter A to the $\div$ A-diet.

Also these animals ceased growing and were killed or died spontaneously, after a shorter or longer duration of the experiment, emaciation, xerophthalmia etc. were developed.

Histologically the finding in these animals was chiefly osteoporosis, but in R 72, R 74, R 77 and R 78 also certain though slight symptoms of rickets, while pure osteoporosis was found in R 73, R 76, R 79 as in R 80 and R 81. These last two animals had had no addition of butter.

The chemical bone examination showed a rather unchanged or perhaps slightly increasing water content.

Even if these two last-mentioned experiments cannot be called quite a success, since the addition of butter proved insufficient to secure a certain growth, the last of the experiments shows that owing to the subminimal addition of butter osteoporosis and the absolute retardation of growth in the bones was not obtained as completely as in animals on an absolutely A-free diet, since the bones still showed symptoms of rickets.

Bone Analyses.

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur			
1) Rats on ÷A-diet, later on N-diet.													
R 47	35	69	28	69	57.8	41.0	51.1	20.9	8.2	18.7	7.7	3.0	7.0
R 48	35	73	35	75	54.7	48.1	49.8	24.0	10.3	18.3	8.8	3.8	5.0
R 49	35	76	39	90	53.7	48.6	50.9	24.7	10.8	16.8	8.2	3.5	1.5
R 50	35	79	34	85	54.7	47.2	50.9	24.0	10.6	18.8	8.9	3.9	2.4
R 51	35	82	37	101	53.9	48.0	52.0	24.9	10.7	18.3	8.8	3.7	6.6
R 52	35	83	34	77	52.2	49.2	53.4	26.3	11.9	19.0	9.3	4.2	5.2
R 53	35	87	38	112	48.9	49.9	50.8	25.4	12.2	18.8	9.4	4.5	5.9
R 54	35	90	36	96	45.4	51.7	50.3	26.0	13.2	18.4	9.5	4.8	6.7
R 55	35	94	35	125	48.7	47.7	52.5	27.0	12.8	17.4	9.0	4.2	3.7
2) Cessation of growth on ÷A-diet, later addition of 0.1 gm butter.													
R 56	30	73	42	73	53.4	44.5	53.3	23.7	10.1	19.8	8.8	3.7	3.9
R 57	30	88	45	57	60.6	42.2	48.8	20.6	8.1	22.2	9.3	3.6	0.1
R 58	30	91	41	60	57.7	43.9	46.7	20.5	8.5	21.0	9.2	3.8	1.3
R 59	30	81	43	70	55.0	44.1	50.1	22.1	9.9	18.9	8.3	3.7	0.4
R 60	30	77	43	46	58.5	42.4	50.3	21.9	8.8	22.4	9.5	3.9	1.5
R 61	30	86	43	68	58.1	41.9	51.7	21.6	8.9	20.4	8.5	3.2	1.9
R 62	30	81	42	53	56.8	42.2	46.8	19.6	8.4	20.1	8.5	3.6	1.8
3) ÷A-diet with an addition of 0.1 gm butter from the beginning of the experiment.													
R 72	45	71	47	85	53.8	47.4	49.8	23.6	10.2	21.3	10.1	4.3	6.5
R 73	45	78	53	85	54.8	42.2	50.7	21.4	8.6	21.4	9.0	3.6	11.2
R 74	45	86	45	61	57.3	43.8	49.1	21.5	8.8	20.4	8.9	3.6	4.0
R 75	45	86	48	78	50.1	45.5	45.6	20.8	8.7	18.8	8.5	3.6	16.0
R 76	45	98	46	89	52.0	48.9	52.2	25.5	11.1	16.6	8.1	3.5	0.0

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur		ash of dry fat free femur	of fresh femur		
R 77	45	107	45	64	55.8	46.5	51.8	24.1	10.6	19.0	8.8	3.9	0.4
R 78	45	103	47	66	52.7	47.6	53.1	25.3	11.4	19.0	9.0	4.1	4.4
R 79	45	103	38	53	58.0	45.3	50.2	22.7	9.4	18.0	8.1	3.4	1.2
R 80 and R 81 had no addition of butter.													
R 80	45	98	39	60	57.6	43.7	48.8	21.3	9.0	18.3	8.0	3.3	0.8
R 81	45	101	55	62	51.8	48.8	51.7	25.2	12.0	18.3	9.2	4.3	1.1

The Influence of a varying Content of Cod-liver Oil in an otherwise A-free Diet.

As it has been maintained in many quarters that butter contains plenty of A-vitamin (growth-promoting) but only a slight amount of »antirachitic vitamin« while cod-liver oil is rich in both qualities, and as it has been proposed to base the most decisive proofs for the assumption of a growth-promoting as well as an antirachitic, not identical, vitamin on these observations, it must be of interest to investigate, if a varying dosage of cod-liver oil would have a similar effect on young rats, especially on their bone formation, as has just been shown in the case of butter.

In an earlier experiment I have shown that lard in our A-free diet could quite well be replaced by pure olive oil. Such an A-free diet also caused cessation of growth in young rats in the course of 4—5 weeks and in the 5th to 6th week incipient xerosis corneae (R 30, R 31 and R 32).

In the experiment I shall now describe, olive oil offers several advantages. Different doses of cod-liver oil are easily dissolved in olive oil, so that a large portion of homogeneous food may be prepared at once, instead of weighing off for the A-free diet certain daily very small amounts of cod-liver oil to be given separately to the animals. In that case the risk is even run that the animals do not eat the addition.

I dared not mix cod-liver oil in our strongly oxidized lard,

as it has been shown by *L. S. Fridericia* (63) that oxidated and tempered fat stuffs may be able to destroy A-vitamin in the diet. As the olive oil was neither tempered nor oxidated, I might suppose that the risk was eliminated.

Furthermore I chose a cod-liver oil that had been kept in a jar in the laboratory for several years, since I supposed that its content of A-vitamin had thus decreased. As further some investigators have tried to prove that old, often oxidated, cod-liver oil may lose its growth-promoting qualities, but still keep its antirachitic qualities, it seemed to me that such a cod-liver oil was bound to be specially fit for these experiments in which I wanted at the same time to examine its influence on growth and on rickets.

I now prepared 6 diet-mixtures according to our usual scheme, and with the usual salt mixture:

- 4.45 gm cod-liver oil dissolved in 218.05 gm oleum olivae (i. e. 2 per cent of cod-liver oil in the fat).
- I ÷A-diet (15 p.c. of olive oil instead of 15 p.c. of oxidated lard) 1000 gm.
- II »0.006 p.c.« of cod-liver oil in the diet (0.0495 gm oil mix.-149.5 ol.o).
- III »0.02 p.c.« of cod-liver oil in the diet (1.5 gm oil mix.-148.5 ol.o).
- IV »0.066 p.c. of cod-liver oil in the diet (4.95 gm oil mix.-145.05 ol.o).
- V »0.25 p.c.« of cod-liver oil in the diet (18.75 gm oil mix.-131.25 ol.o).
- VI »2 p.c.« of cod-liver oil in the diet (150 gm oil mix.-0 ol.o).

16 young rats: R 82—R 90 inclusive of the same litter and R 91—R 97 of another litter were when respectively 33 and 31 days old, put in experiment. Each diet mixture was tried on three rats, however, there was only one rat left for the diet with »2 p.c.« cod-liver oil.

Now it turned out that the different concentrations of cod-liver oil, like the different additions of butter caused a difference

in the weight curves of the rats. One part showed cessation of the rise of the weight curve, and after about five weeks' feeding even loss of weight, while the other part showed an uninterrupted rise of weight. It further turned out that the diets with less than »0.25 p.c.« of cod-liver oil were unable to produce a sure rise of the weight.

The diet mixtures IV, III, II and I caused not only cessation of the rise of the weight curve, but the occurrence of xerophthalmia, abscess of the root of the tongue, cessation of growth, and in all respects the same as we saw earlier, with feeding with a pure ÷-A-diet and with the experiment with butter, when the addition of butter proved insufficient to secure the growth. The animals were killed 80 days old.

Upon autopsy the findings in rats of group I were xerophthalmia, emaciation. Slight rosary in R 82 and R 83. No symptoms of rickets in R 91.

In group II, xerophthalmia, emaciation and abscess of the root of the tongue. In R 84 some fractures of costae, but no rickety symptoms in R 92 and R 93.

In group III, xerophthalmia and symptoms of rickets in R 86 and R 94, but no rickety symptoms in R 85.

In group IV, xerophthalmia and rosary in R 87, R 95, and R 96.

In group V; macroscopically nothing abnormal in R 88, R 89, and R 97, nor in R 90 from group VI.

The histological examination in group I showed: in R 82 some osteoporosis, slight symptoms of rickets with irregular cartilage proliferation zone, extended provisional calcification zone and osteoid tissue. In R 83, some osteoporosis and slight symptoms of rickets. In R 91, osteoporosis, arrested growth in the proliferation zone, no irregularity of the cartilage cells, but some osteoid tissue in the spongy trabeculae.

Group II: R 84, R 92, and R 93 all showed osteoporosis, but also distinct symptoms of rickets with irregular cartilage proliferation zone, extended provisional calcification zone and some osteoid tissue. Group III: (R 85, R 86, R 96) and group IV (R 87, R 95, and R 96) all showed some osteoporosis, but also

distinct rickets, the osteoporosis, however, was most marked in R 96.

In group V, R 88 showed distinct rickets with osteoid tissue, irregular cartilage proliferation zone, extended calcification zone, some osteoblasts and hardly any osteoporosis. R 89, showed similar findings, but slighter amounts of osteoid tissue, and R 97 showed almost normal conditions.

Group VI: R 90 showed normal bones.

This means that experimental rickets can still be produced on a diet of »0.25 p.c.« of cod-liver oil.

The chemical bone analyses showed facts similar to those seen in the experiments with butter: bones hydrous and poor in minerals in animals where the growth had ceased and less hydrous and richer in minerals when the growth was secured.

From this it appears that the curative influence of cod-liver oil on 1) xerophthalmia and osteoporosis, arrested growth, 2) on arrested or some growth, in which the animals do not show xerophthalmia, but distinct changes of the skeleton, 3) on a normal rise of weight and normal development of bone, may in all circumstances be explained as a question of dosage.

The Influence of an A-free and deficient Phosphorus Diet with varying Content of Cod-liver Oil on the Growth and Formation of Bone in young Rats.

In order once more to elucidate and deepen the knowledge of the importance of mineral salts for the development of rickets the following experiments were carried out.

For these experiments were used 12 young rats. Owing to certain objectionable circumstances in connection with our rat-stud, it was impossible to procure one or two litters fitted for the experiment at this time, so I had to use 12 young rats, as far as possible of the same weight (33—56 gm), without having any knowledge of litter or exact age.

The diet mixtures for this experiment I produced in the same

way as before, but with salt mixture III instead of our usual salt mixture. As the slight amounts of cod-liver oil had proved to offer little advantage over a pure $\div$ A-diet, I omitted the mixture corresponding to II (≈ 0.006 p.c.« cod-liver oil) in the preceding experiment and instead produced a mixture with ≈ 4 p.c.« of cod-liver oil. In this way I thought I was on the safe side, if it should prove that ≈ 2 p.c.« in this experiment should be insufficient to cause normal bone development.

As will be seen from the weight curves, a stand-still occurs in the animals of group I (R 98 and R 99), also in group III (R 100, R 101) and in group IV (R 102, R 103), while the animals in the groups V, VI and VII (≈ 4 p.c.«) show a continual rise of weight. R 105 of group V died early from intercurrent disease. The animals in I, III and IV showed xerophthalmia and some macroscopical symptoms of rickets, while autopsy of animals of V, VI and VII did not macroscopically show anything abnormal.

Consequently in this case, too, the limit for preserved growth was just under ≈ 0.25 p.c.« of cod-liver oil in the diet.

Histologically was found in group I: Rat 98 some osteoporosis, some preserved cartilage cells in the provisional calcification zone, which was deficiently calcified, there was distinct rickets and some osteoid tissue. R 99: osteoporosis was the most prominent symptom, only slight symptoms of rickets, thin osteoid borders.

III, R 100 and R 101 both showed some osteoporosis and distinct rickets.

IV: R 102 showed rickets with widely enlarged zone of big clear non-differentiated cartilage cells, here and there with irregular limit towards the marrow cavity. Such an accumulation of cartilage cells was found in several of these animals on $\div$ A $\div$ P-diet, and seems especially in a high degree to dominate the picture of the experimental rickets of *McCollum* on a calcium-rich and phosphorus-poor diet.

R 103 showed osteoporosis and dubious rickets.

V: R 101 some osteoporosis, but otherwise nearly normal ossification zone.

VI: (R 106 and R 107) and VII: (R 108 and R 109) showed normal bones.

The chemical bone analyses showed facts corresponding to earlier experiments.

The Influence of a $\div A \div Ca$ -diet with varying Content of Cod-liver Oil on the Growth and Bone Formation of Rats.

This diet was produced in the same way as the preceding one, but with salt mixture II, instead of our normal salt mixture.

For the experiment were used 12 young rats 28 days old, belonging to the same litter. All the animals were killed when 70 days old.

Also in this experiment the growth of the animals showed clearly the inhibitory influence of the deficient calcium in the diet.

It will be seen from the curves that only in group VI and group VII do the animals show (R 118 and R 119 — R 120 and R 121) a secured rise of weight, thus the content of cod-liver oil (≈ 0.25 p.c.) in this diet does not guarantee a rise of weight. Xerophthalmia is developed in animals of group I and III, but is not surely present in group IV. Fractures of the ribs are still found in group V.

Histologically osteoporosis was found in all the bones of these animals, but further distinctly rickety symptoms in the epiphyseal line with preserved non-differentiated, clear cartilage cells often piled up on each other in the epiphyseal line. The cartilage matrix is uncalcified. There are only slight amounts of osteoid tissue. First group VI: (R 118 and R 119) and VII: (R 120 and R 121) apart from some rarefied bone tissue with few and thin spongy trabeculae and thin compacta, showed normal ossification processes.

The chemical bone analyses showed an extraordinarily high content of water in the bones of these animals and a very low Ca and P content even in the animals receiving ≈ 4 p.c. of cod-liver oil added to the diet.

From these investigations it appears that with a deficiency of Ca and P in the diet the histological picture of rickets apparently gets more pronounced if there is at the same time a deficit of A-vitamin, and the bone rarefaction, the osteoporosis, gets more dominant. The bones get softer; more hydrous and containing less minerals. It is also seen from the chemical analyses that 4 p.c. of cod-liver oil in the diet is not sufficient to give as good ossification, as 2 p.c. in a diet having an adequate salt content. It is also seen that such animals (from the phosphorus-poor groups VI and VII) which, on account of a greater dose of cod-liver oil have grown more rapidly than animals with a smaller dose of cod-liver oil, have no essentially lower water content or greater ash content than the animals which, on account of lower doses of cod-liver oil, have grown slower. This is seen even more distinctly on considering the animals in the calcium-poor groups V, VI and VII.

Thus it has been shown that with increasing doses of A-vitamin added to the diet increasing amounts of minerals are retained in the osseous system. Further it has been shown that a considerably larger A-vitamin content in the nourishment is required, if the growing organism is to be protected against a relative deficiency of minerals in the nourishment. Furthermore, it has been shown that an increased supply of A-vitamin is but a gain for the growing organism, if the administered amount of salt is adequate and sufficient.

Bone Analyses.

No. of rat	Age in days at the		weight at the		H_2O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur			
1) ÷ A-diet with different content of cod-liver oil.													
R 82	33	83	50	85	55.0	44.7	51.3	23.0	8.8	20.5	9.2	3.5	14.3 I
R 83	33	83	47	75	54.5	46.5	52.9	24.6	10.2	18.5	8.6	3.5	8.7 »
R 91	31	78	51	73	55.8	43.3	54.0	23.4	8.7	20.4	8.8	3.3	15.3 »
R 84	33	83	53	86	54.9	52.1	45.4	23.7	9.5	16.6	8.6	3.5	10.5 II
R 92	31	78	45	75	53.5	45.4	54.3	24.6	9.6	20.4	9.2	3.7	14.1 »

No. of rat	Age in days at the				weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent				per cent fat
	beg.		final		beg.				final		ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur		
R 93	31	83	40	66	55.1	44.1	55.8	24.6	9.2	20.4	9.0	3.3	16.1	»		
R 85	33	74	48	84	53.3	43.6	50.1	21.9	9.4	21.5	9.4	4.0	8.1	III		
R 86	33	83	55	82	55.0	45.0	53.0	23.8	9.3	20.0	9.0	3.5	12.1	»		
R 94	31	78	49	73	49.9	46.8	53.3	25.0	10.3	20.5	9.6	3.9	17.9	»		
R 87	33	83	48	87	52.8	49.0	52.7	25.8	10.8	18.9	9.2	3.8	11.2	IV		
R 95	31	78	48	77	52.8	47.8	52.2	25.0	9.8	20.4	9.7	3.8	16.6	»		
R 96	31	78	45	65	51.3	45.7	51.6	23.6	8.8	21.4	9.9	3.7	23.0	»		
R 88	33	83	55	126	50.1	50.4	52.0	26.2	11.2	18.6	9.4	4.0	14.5	V		
R 89	33	83	55	124	49.9	50.1	53.1	26.6	11.3	18.9	9.5	4.0	15.4	»		
R 97	31	78	45	113	47.6	53.2	53.6	28.5	12.7	18.1	9.6	4.3	14.8	»		
R 90	33	83	48	146	47.2	57.8	52.0	30.1	15.2	17.4	10.1	5.1	4.4	VI		

2) ÷ A ÷ P-diet with different content of cod-liver oil.

R 98	33?	79?	54	84	54.3	44.1	52.0	22.9	9.1	20.4	9.0	3.5	12.8	I	
R 99			39	71	55.1	40.0	57.1	22.8	8.0	22.9	9.1	3.2	21.3	»	
R 100			48	86	58.1	40.0	51.1	20.5	7.5	19.9	7.9	2.9	12.4	III	
R 101			35	73	56.3	40.5	53.4	21.6	7.3	24.0	9.7	3.2	22.7	»	
R 102			56	117	53.2	44.6	51.1	22.8	9.4	20.7	9.2	3.8	8.0	IV	
R 103			37	59	54.4	41.8	55.7	23.3	8.6	22.3	9.3	3.4	19.0	»	
R 104			54	123	50.5	50.4	49.7	25.0	11.4	19.0	9.5	4.3	8.0	V	
R 106			56	147	49.8	51.4	52.3	26.8	12.2	18.2	9.4	4.2	8.9	VI	
R 107			33	88	49.2	49.4	50.2	24.8	10.8	18.7	9.2	4.0	14.4	»	
R 108			55	124	49.0	52.2	50.4	26.3	12.3	19.4	9.7	4.5	6.4	VII	
R 109			56	89	51.1	49.0	51.3	25.1	11.1	20.8	10.2	4.5	9.7	»	

3) ÷ A ÷ Ca-diet with different content of cod-liver oil.

R 110	28	70	30	39	63.5	36.5	51.5	18.8	6.7	24.8	9.1	3.2	1.7	I	
R 111	28	70	48	72	63.9	34.2	49.9	17.1	5.9	24.5	8.4	2.9	4.0	»	
R 113	28	70	46	67	63.9	33.4	49.3	16.5	5.6	25.2	8.4	2.8	5.1	III	
R 114	28	70	30	44	59.3	41.3	50.6	20.9	7.9	25.8	10.6	4.0	6.4	IV	
R 115	28	70	42	64	59.1	40.5	48.4	19.6	7.3	30.1	12.2	4.5	8.1	IV	
R 116	28	70	28	40	59.7	38.7	45.4	17.6	6.2	28.8	11.1	3.4	12.2	V	
R 117	28	70	30	46	59.9	41.7	49.1	20.5	7.3	23.6	9.8	3.5	10.8	V	
R 118	28	70	43	89	60.7	41.7	50.2	21.0	7.6	22.3	9.3	3.3	7.6	VI	
R 119	28	70	28	55	71.7	37.8	52.6	19.9	4.9	25.0	9.5	2.3	12.6	»	
R 120	28	70	28	70	57.4	40.6	49.5	20.1	8.0	21.6	8.8	3.5	6.1	VII	
R 121	28	70	30	91	58.5	41.6	50.0	20.8	8.3	24.1	10.0	4.0	3.0	»	

Average.

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent of fresh femur	P per cent of fresh femur	Diets
	beg.	final	beg.	final					
÷A-diet with different content of cod-liver oil.									
3	33	81	49	77	55.1	44.8	9.2	3.4	I
3	32	81	46	75	54.5	47.2	9.4	3.5	II
3	33	78	50	79	52.7	45.1	9.6	3.8	III
3	32	79	47	76	52.3	47.5	9.8	3.8	IV
3	33	81	51	121	49.0	51.4	11.7	4.1	V
1	33	83	48	146	47.2	57.8	15.2	5.1	VI
÷A ÷P-diet with different content of cod-liver oil.									
2	35?	79?	46	77	54.7	42.0	8.5	3.3	I
2			41	79	57.2	40.2	7.4	3.1	III
2			46	88	53.8	43.2	9.0	3.6	IV
1			54	123	50.5	50.4	9.5	4.3	V
2			44	114	49.5	49.9	11.5	4.1	VI
2			55	106	50.0	50.6	11.7	4.5	VII
÷A ÷Ca-diet with different content of cod-liver oil.									
2	28	70	39	55	63.7	35.3	6.3	3.0	I
1	28	70	46	67	63.9	33.4	5.6	2.8	III
2	28	70	36	54	59.2	48.9	7.6	4.2	IV
2	28	70	29	43	59.8	40.2	6.7	3.4	V
2	28	70	35	72	65.7	40.0	6.2	2.8	VI
2	28	70	29	80	57.9	40.1	8.1	3.7	VII

These last three experiments seem to show that there is some reason to suppose that a Ca- or P-poor diet causes deeper changes of the osseous system, than if the salt content had been adequate, and that a somewhat higher content of A-vitamin is required in such a salt-poor diet for protecting the organism against the injurious influence of this diet.

Histologically, too, there seems to be some difference between the rickets produced in these experiments and the rickets produced in the experiments with butter and with an adequate salt content. The large accumulation of cartilage cells in the proliferous zone and in the zone corresponding to the provisional

calcification zone is rather conspicuous in these animals. The cartilage cells are big and clear, have, as it were, a more airy look and the matrix is so to speak quite uncalcified. This must no doubt be explained as a result of the more pronounced growth-inhibitory and growth-stopping qualities of the diet. The cartilage cells remain in a still less differentiated, still more »primordial« stage, than during the usual $\div$ A-experiments with an adequate salt content. The accumulation is brought about by the cartilage cells lying nearest the resting cartilage cells being the last to cease growing. This fact may almost be seen from the histological pictures, but is also supported by numerous observations, that may serve as analogous. Thus the primordial egg cell in the ovary proves much more resistant to the injurious influence of X-rays than more differentiated cells, *Graaf's* follicles, corpus luteum cells etc. and the primitive cells in testes more resistant than the spermiocytes of the first and second order, not to speak of the spermatids.

These observations also explain why the histological picture of *McCollum's*, *Pappenheimer's* and other investigators' experimental rickets is apparently so impressive. As known they use a vitamin-poor diet which moreover is extraordinarily insufficient as regards salts, especially phosphorus, and hereby they are able to produce an extremely widened, broad, rather even, thickened epiphyseal line, while the osteoid tissue is not very marked. Here the cartilage cells also have the immature, non-differentiated »airy« character. This »airy character« not only vanishes by the addition of cod-liver oil, but also by replacing $\frac{1}{7}$ of the calcium carbonate by 0.4 per cent of $K_2 HPO_4$, furthermore by starvation for three days and by radiation. This phenomenon all earlier investigators call healing of rickets, as most of them have observed that simultaneously slight amounts of calcified substance appear in the widened epiphyseal line. While with the addition of cod-liver oil it is exceedingly likely that healing commences immediately, it is very doubtful in the case of the three last-mentioned measures, and probably it is a question of mere resorption of these non-differentiated cartilage

cells, by which the severe osteoporosis in the bone becomes more apparent, while growth in the proliferous zone is not observed.

*Does a Growth-Promoting and an »Antirachitic« Vitamin exist?
Or are they identical?*

Having thus in the preceding experiments thrown light on and examined that vitamin in the diet which causes rise of weight, growth, prevention of xerophthalmia and healing or prevention of rickets, and having in all these experiments not only seen a parallelism in these qualities, but even such a close connection between them that they must be supposed to be identical, I shall now enter more closely upon such experiments as attempt to show that the anti-xerophthalmic and growth-promoting quality is associated with another principle than the antirachitic quality.

Originally the term »fat-soluble vitamin« was used for the component bound to certain fats, but not exactly delimited, which, if lacking in an otherwise satisfactory diet, causes cessation of growth in young rats, and if growth has thus ceased, is again able to produce growth when these fats are added to the diet.

Later it was observed that the deficiency of these substances in the diet caused development of xerophthalmia in the experimental animals, for which reason the supposed vitamin was called anti-xerophthalmic vitamin. Or as it was believed that these effects were of a different nature, it was even supposed, that there existed a growth-promoting A-vitamin as well as an anti-xerophthalmic vitamin (C. Funk (68)).

As furthermore it had been gathered from the studies on rickets that these components of the fats play a very essential rôle for the deposition of calcium in the bones, and as it was thought possible to conclude from several experiments that this calcium-regulating quality differed as much from the growth-promoting as the anti-xerophthalmic quality, and as it had even

been believed to be proved that a substance may be deficient in the two last-mentioned qualities, but still be in possession of the antirachitic quality, it was maintained that most likely there exist two fat-soluble vitamins.

Against this speaks partly the simultaneous presence of these vitamins in the same substance, thus in cod-liver oil, milk-fat and yolk of egg. Partly it has been shown that food stuffs deficient in the growth-promoting vitamin are also deficient in the anti-xerophthalmic and anti-rachitic qualities, thus olive oil, lard and others.

Partly numerous experiments for the purpose of delimiting and determining the vitamin: experiments of saponification by *Drummond & Coward* (44), *Zucker, Pappenheimer & Barnett* (278), *Zucker* (276) a. o. have shown that these qualities are all found in the unsaponifiable remainder of certain fats, but that the effect may be destroyed by oxidation. *Harry E. Dubin* (46) shows that the unsaponifiable fraction corresponds exactly in effect to cod-liver oil and *E. Poulsson* (195) finds that 0.004 mgm of unsaponifiable residuum has the same growth-promoting power as 2 mgm of cod-liver oil.

It has been maintained that the occurrence of antirachitic vitamin may be different from the occurrence of growth-promoting vitamin. Thus *Mellanby* has believed he was able to show that vegetable oils have a graduated content of antirachitic qualities. These are supposed to be found in greater amount in peanut and coco-nut oil, less in rape, cotton-seed, olive and linseed oils. Lard is even supposed to have an antirachitic effect. As it has been proved that coco-nut oil does not possess anti-xerophthalmic (*McCollum* and many others), but antirachitic qualities (*Mellanby*) and as it has been thought possible to conclude from the experiments of *McCollum, Simmonds, Becker & Shipley* (169) and *H. Steenbock & M. T. Nelson* (224) that butter only in a slight degree acts antirachitically, but is rich in anti-xerophthalmic vitamin, it has been thought possible in this to see a proof that these qualities depend on two different substances.

This supposition was further supported by numerous experi-

ments by *McCollum, Simmonds, Becker & Shipley, Goldblatt & Zilva* (73) a. o. These investigators thought they were able to prove that the antixerophthalmic effect of cod-liver oil was destroyed by oxidation at 100° C. for 12—28 hours, but that the antirachitic effect was still preserved. Similar facts have been found by *Glanzmann & Sieffert* (69) by investigations on cod-liver oil which had been blown through with oxygen for 3 hours, furthermore by oxidation in a waterbath for 3—4 hours during frequent shaking with 30 per cent H_2O_2 . Hardening had a similar effect (action of hydrogen on cod-liver oil at 250° C.).

To all these conclusions drawn from earlier experiments must now be raised the objections that I have already previously mentioned.

Owing to the different experimental diets used to produce rickets by the different investigators the results cannot be directly compared. When thus *Mellanby* thinks he has proved that coco-nut oil possesses a relatively high antirachitic action, it must be remembered that his diet always contains more or less milk or skimmed milk, which contains some vitamin, more in the summer than in the winter. If he now adds a vegetable oil which is vitamin-free, he can obtain a decrease of the rickety changes of the bones, and this cannot be understood as healing, but on the contrary as osteoporosis of the bones, and as he could not prove derangement in the cartilage growth zone and osteoid tissue, he believed that the bone was normal.

The same facts appear in a very marked degree in the experiments of *McCollum, Glanzmann & Sieffert, Pappenheimer* a. o. As a matter of fact it may also be seen from many of the microphotos which accompany their papers, especially in the case of the experimental rickets, where the accumulation of the cartilage cells have the »airy character«, which characterizes rickets on an A-poor and phosphorus-poor diet. To this must be added that many of the investigators have not even used the histological method of investigation, they have been content with »the line test«, the uncertainty of which as well as its dependence on many other factors have also been mentioned. Furthermore some have used the P-content of serum as diagnosticon, but this,

too, is dependent on unknown factors, whose action is not yet clear (starvation, light etc.).

In no case on a purely synthetic diet has it been proved by these experiments that a substance acts antirachitically without simultaneously being growth-promoting, not even in experiments with radiation. In these last experiments the investigators have sometimes succeeded in making the weight curves of the rats rise a little for a few weeks (3—5 weeks), but then a considerable loss of weight and emaciation occurred (*Powers, Park & Simmonds* (197), *E. M. Hume* (123), *Wallman & Vagliano* (264)). Many investigators have observed that in bones from such animals no rickets was found, they even assert that the bones are normal, for which reason they think the measures used have acted antirachitically. *Wilhelm Stepp & Ernst Woenckhaus* (250) have examined the influence of different lipoids added to the rachitogenic diet No. 3143 of *McCollum*. Thus they examined lecithin, cholesterol, cephalin, cerebrosin, withdrawing one at a time. They then found that lecithin and cholesterol are without importance for the bone formation, in spite of the animals not growing at all on this diet and being in all respects very slack. Only at the end they introduce a little remark about the non-appearance of rickets perhaps being due to the cessation of growth.

But they entirely forget that all investigators have maintained that growth was necessary for the appearance of rickets, they forget that a rickety bone picture may disappear by starvation and the thought never occurs to them that a rickety bone from which the organism itself resorbs the organic components (osteoid tissue, proliferated cartilage cells) will not be a normal bone, but precisely osteoporotic. This might have been avoided, if they had undertaken partly the obligatory histological examination of the bones partly the obligatory chemical examination. They would then have seen that such bones, which they call normal, are more hydrous than normal bones of the same weight and size, let alone, in normal animals of the same age.

The numerous experiments which group themselves around these questions may, even if individual authors have apparently

hardly delimited the separate, possibly essential, differences, be divided into

1) such experiments which attempt to show that direct radiation of the skin of the experimental animals has the effect that animals on a rachitogenic diet escape rickets or, if rickets is already produced, that they are healed by radiation.

2) partly experiments which in accordance with the views of *Hess* and Co-workers and *Steenbock* a. o. attempt to prove that radiation with ultra-violet rays imparts to different organic substances and vegetable oils (cotton-seed oil, olive and linseed oil, cholesterol and phytosterol, salad, spinach, wheat, hay, flour and milk, and dry-milk) antirachitic qualities, whether these were absent beforehand or only present in a slight degree.

3) partly experiments attempting to show that direct radiation may produce a synthesis of antirachitic vitamin in the normal organism.

4) partly experiments which on the basis of the theory that it is the ultra-violet rays, *sui generis*, which act as an antirachitic principle, try to show that all foods acting antirachitically emit ultra-violet rays and that these condition the antirachitic action.

5) As it was proved later that antirachitic substances do not emit any sort of rays, but that certain of these substances by being placed directly under a photographic membrane showed ability to influence this and, as it was believed to have been proved that these substances did not possess any growth-promoting ability, but antirachitic qualities, it was thought that here was a proof that these qualities were not due to a common principle.

ad 1) While some of the investigators believed they had proved that the growth of rats on an A-vitamin-poor diet might be stimulated by radiation with mercury-vapor light (*E. M. Hume & M. H. Smith* (125)), all later investigators could not confirm this view. Neither could *Webster & Hill* (265), *A. F. Hess* (89), *G. F. Powers*, *E. A. Park & N. Simmonds* (197) prove any rise of weight in such rats by exposing them to sunlight or ultra-violet rays. *E. Wallman & M. Vagliano* (264) could not

prove a quicker growth in radiated rats on a normal diet, than in non-radiated animals.

On the contrary numerous investigators maintain that a direct radiation of the experimental animal may be the cause why animals on a rachitogenic diet avoid getting rickets, or are healed if rickets has already occurred (*A. F. Hess & L. I. Unger* (105), *P. Schultzer & Sonne* (222)). As previously mentioned, this has likewise been maintained by numerous clinicians, *Huldschinsky* (121, 122), *J. Jundell* (132, 133).

Thus *C. E. Bloch & Frans Faber* (20) and *C. E. Bloch* (19) maintain that neither ultra-violet rays nor sunlight can heal xerophthalmia or replace the A-substance in the animal organism, but that the energy of the ultra-violet rays may be supplied to the organism partly directly by radiation, partly by certain articles of food in which they suppose this energy is stored, e. g. cod-liver oil, yolk of eggs, and to a certain extent milk, and thus act antirachitically. According to this conception the light-therapy must be a treatment of causes and the administration of cod-liver oil a substitution-therapy.

ad 2) Most of the American and English investigators have hardly thought, however, that the effective antirachitic principle is a form of light-energy. They suppose that radiation is able to communicate the antirachitic qualities to certain substances (vegetable oils, milk, dry milk, cholesterol etc.) or render them active, (*H. Steenbock & A. Black* (237), *Hart, Steenbock & Elvehjem* (84)). *Steenbock & Black* (238) maintain that the radiation of certain articles of food and of the unsaponifiable fraction of the fats causes these to get both a growth-promoting quality and the ability to increase the calcification in the bones of rats. It is easily seen, though, that the rise in weight which can be produced, is very small and only lasts 3—4 weeks. *Wallman & Vagliano* (264) and *Lesné, R. Turpin & P. Zizme* (151) could not prove a rise in weight or a greater deposition of calcium in the radiated rats than in the non-radiated.

A. F. Hess & Mildred Weinstock (93, 94) maintain that an article of food to acquire an antirachitic effect by radiation

must contain cholesterol or its derivatives. They believe that it is these substances which are transformed by radiation to antirachitic substance, and they base this supposition on the fact that the absorption spectrum of cholesterol is changed by radiation. Thus it is supposed that it is not a physical property which is imparted to the substance, but that a real chemical transformation takes place. Similar facts have been shown by *Frederick W. Schultz & M. Morse* (219), *A. Hess, M. Weinstock & F. D. Helman* (100), *H. Steenbock & A. Daniels* (240), *A. F. Hess & M. Weinstock* (95), *H. Steenbock & M. T. Nelson* (245).

Also *P. György & P. Popoviciu* (77) try to prove that radiated cod-liver oil, emulsion of cod-liver oil, radiated salad and olive oil, radiated rat-skin and radiated cholesterol and cholesterol-ether act antirachitically. All these investigators use either *McCollum's* diet No 3143 or *Sherman—Pappenheimer's* No 84. *György & Popoviciu* thus used the latter for rats. They have judged of the results by macroscopic bone examination and röntgenographically, and in some cases they have determined the Ca content in dry, extracted bone. The duration of the experiments was only 5—6 weeks. It must be noted that the animals getting radiated cod-liver oil added to the diet did not increase in weight and did not behave differently to animals with an addition of non-radiated olive oil. The animals getting an addition of non-radiated rat-skin have risen a little in weight and about these animals the authors remark that they have typical rickets. On the contrary, the animals getting radiated olive oil, radiated rat-skin, radiated cholesterol etc. mostly showed a certain loss of weight, and in the bones of these animals rickets was not proved. Therefore *György & Popoviciu* as well as the above-mentioned American investigators maintain that the non-radiated fats are absolutely ineffective against rickets, but that by radiation they are rendered active as antirachitic substances.

When it is seen that non-radiated rat-skin gives some, even though slight growth, and it is known (*Skuli V. Gudjónsson* (75)), that there is reason to believe that the skin is the part of the organism which contains relatively most vitamin, it ought to have been understood why these animals showed typical rick-

ets, while animals getting radiated rat-skin show rickets no more, probably because the vitamin content is destroyed by the radiation. And in these last-mentioned animals the bones are likely to be typically osteoporotic.

Charles E. Bills & Francis G. McDonald (9) mention having produced a polymerisation product (tricholesterol) by treating the cholesterol with phloridzin. They state that this is not antirachitic nor can it become so by radiation, but that a continued influence of phloridzin may produce this quality. This has also been tried on *McCollum's* diet No 3143, and after 5 days of experiment judged of by »the line test«. All the animals lost weight.

All investigators now agree that a synthesis of A-vitamin cannot be obtained by radiation. But as we have seen, most of them maintain that it has been proved or made probable as regards the antirachitic factor, thus also *J. Serebrijski*, *H. Vollmer & E. Zadeck* (223), *A. F. Hess*, *M. Weinstock & E. Sherman* (101).

ad 3) As all these experiments are occupied with a synthesis produced by radiation in certain organic matter, especially cholesterol and its derivatives, it has been thought that the effect of the ultra-violet light bath on the organism must be due to the epidermis containing a great amount of cholesterol in the deeper layers and that this is rendered active by ultra-violet radiation and thus acts antirachitically. This theory has been held by *O. Rosenheim & J. C. Drummond* (204), *O. Rosenheim & A. Webster* (205), *H. C. Eckstein & Udo J. Wile* (50), *A. F. Hess & M. Weinstock* (92).

An attempt has been made to support his view by pointing out that ultraviolet light has great ability to penetrate the skin of living rabbits, dogs, and cats. (*D. I. Macht, F. K. Bell & C. F. Elvers* (158)). Further it has been attempted to prove that radiation of a mother-animal caused the milk to be more vitamin-containing. This *E. M. Hume* (124) did not succeed in proving conclusively, as the cows which were under sunlight at the same time got fresher and better pasture than the stall-fed cows, which were kept in darkness. *E. M. Luce* (156) also found that

the content of A-vitamin in cow's milk determined by growth experiments is independent of whether the cow is exposed to sunlight or is in a dark stable, while she thinks she is able to show that the antirachitic properties of the milk (determined by *McCollum's* diet) increased by the cow being exposed to sunlight.

A. F. Hess (89), *A. F. Hess*, *M. Weinstock* & *E. Sherman* (102) have thus thought they could prove that ultra-violet radiation of a mother or nurse causes the milk to become highly antirachitic. Similar results *H. Steenbock*, *E. H. Hart*, *C. A. Hoppert* & *A. Black* (241) believe to have obtained by radiating a goat. But to judge of the effect they all used *Sherman—Pappenheimer's* regime which is inadequate for this purpose.

It has even been attempted to reduce the importance of a vitamin-rich diet to pregnant and nursing mothers. *Hess* & *Weinstock* have tried conventional administration of cod-liver oil to mothers during lactation and during the two last months of pregnancy, and found that the children were not prevented from getting rickets. *E. M. Hume* proved that the vitamin content of the mother milk only increased if the mother was in a good state of nutrition, in the opposite case it seems as if the mother herself consumes the A-vitamin.

Richard Wagner & *Hans Wimberger* (263) examined the influence of great doses of cod-liver oil on milk-cows. They found that the milk from such animals fed on cod-liver oil had some healing effect on rickets in children, but *E. M. Luce* could not show any specially antirachitic quality in this milk, which had unfortunately been sent to London in a pasteurized state to be examined there. *Wagner* & *Wimberger* therefore maintain that the antirachitic vitamin can hardly be transferred to the milk by feeding.

That light should be the essential and determining factor as to whether a substance possesses antirachitic qualities cannot, however, be confirmed by any other observations from the vegetable and animal kingdoms. Thus *A. F. Hess* & *M. Weinstock* (96) have shown that a puffer-fish (*spheroides maculatus*), which has a great content of vitamin, is as well able as

ever to store antirachitic vitamin when it has been deprived of sunlight and ultra-violet light for a long time, it is only the nature of the nutrition, which is determining for the content of A-vitamin in the fish. Similar facts may no doubt be shown in the case of the cod-fish; this fish does not, as is known, live at the surface of the water. Furthermore it is proved that germinating wheat can synthesise vitamin even if the germination takes place in darkness. *Coward & Drummond* have maintained that A-vitamin can only be formed during germination if certain photo-chemical processes take place which have been thought to be connected with the formation of chlorophyl. *Wilhelm Stepp* (247, 248) shows that the production of A-vitamin is independent of the chlorophyl, while he thinks he can draw the conclusion that no antirachitic vitamin is produced during germination.

Also this last conclusion is based on the uncertain results which can be drawn from the experiments with young rats on *McCollum's* diet No 3143 and »the line test« method.

Nay, things have even gone so far that it has been proposed and already tried to treat children with an irradiated diet or radiated food articles, and perhaps this will be continued until it appears that in this way xerophthalmia may be produced in the children. Only then perhaps will it be remembered that all the experiments have shown that radiation is able to destroy the content of the growth-promoting vitamin in the food.

Thus *Benjamin Kramer* (146) has treated rickety children with milk which had been radiated for two hours; later he found that 10—20 minutes' radiation with ultra-violet light was sufficient. He maintains that healing of rickets occurred (ascertained röntgenologically and by the Ca and P content of the blood). Nothing is said about the weight of the children.

H. Beumer (8) could not observe any effect on rickets in children by treating them with 0.15 gm of radiated cholesterol per diem.

P. György has tried to use »jekorisierte Milch« against rickets and maintains that milk radiated for half an hour to one hour with mercury-vapor light, during stirring, has become plainly antirachitic.

Amy L. Daniels, Sarah Idell Pyle & Lela Brooks (32) even try to show that radiated winter milk is able to produce better growth for young sucking rats. These experiments are little convincing, however, partly many different litters of rats are in question and partly the mother animal receives a very mixed diet, in which is even found cod-liver oil. The cod-liver oil has perhaps been of varying quality.

ad 4) The aim of numerous of the experiments has been to try if ultra-violet rays issue from such substances as are known to be highly antirachitic, viz. cod-liver oil, yolk of egg, the unsaponifiable fraction of cod-liver oil etc.

These investigations were first undertaken by *Kugelmass & McQuarrie* (147). In the first experiments they thought they were able to prove that antirachitic substances emit ultra-violet rays i. e. rays which can pass through quartz, but not through glass, and which, after this passage, can influence a photographic plate. They found that this plate-blackening effect is increased by oxidation of the substances, while it is weakened by reduction. Substances that are not antirachitic (linseed oil, arachis oil, lard etc.) do not blacken the plate even after oxidation, unless there is a free space without covering-glass or quartz plate between the substance and the photographic membrane.

Later investigators, *A. Webster & L. Hill, Steenbock* and others show that substances with antirachitic action do not emit ultra-violet rays. But as also shown by *Malmström* (160) fats which have been radiated with ultra-violet light, may produce blackening of a photographic plate which is placed above the fat. This influence cannot take place if a glass or quartz plate has been pushed in between the fat and the photographic membrane. He therefore assumes that by the ultra-violet radiation are produced certain oxidation processes in the irradiated substance and that this is hereby able to send forth gasses which may influence a photographic plate. This has been thoroughly tried and proved by *H. Harthausen* (87) and by *Eigil Reckling* (201), and they both suppose that the phenomenon is due to processes of oxidation, produced in the radiated substances.

In spite of the fact that several substances which are beyond

all doubt antirachitic (cod-liver oil), have been shown by several investigators (*Frederick W. Schultz & M. Morse* (219)) not to possess any photo-activity, but only to obtain this activity by standing over and by oxidation, and in spite of the fact that the growth-promoting vitamin is destroyed thereby, the investigators believe that the antirachitic quality has been preserved, and in the numerous experiments with cholesterol and its derivatives has even been developed in such substances where this quality was not present before (*A. F. Hess, M. Weinstock & D. F. Helman* (100), *H. Steenbock & A. Daniels* (140), *A. F. Hess & M. Weinstock* (75), *H. Steenbock & M. T. Nelson* (245)). Hence this phenomenon of oxidation has been the all-pervading one in their conception of the antirachitic substances. Thus also *H. Goldblatt & S. S. Zilva* (73) maintain that oxidated cod-liver oil, even if it has lost its growth-promoting power in this way fully possesses the antirachitic power. The different authors all find that the effective rays lie between 210—300 $\mu\mu$ (*Hess & Weinstock* (97), *P. G. Shipley* (225), *P. Schultzer & C. Sonne* (222)).

On all these experiments on the influence of ultra-violet radiation of animals and food-stuffs or oxidation processes thereof the theories of the different aetiology of rickets and xerophthalmia are based, as well as of the growth factor. But all the experiments suffer from the drawbacks previously mentioned: The experiments are carried out on calcium-rich and phosphorus-poor diets with a variable but insufficient content of vitamin. The experiments are of too short duration (4—6 weeks). As diagnostical methods are used only »the line test« or the phosphorus content of the blood. When the content of ash or calcium in the bones has been examined, it has only been given in relation to dry fat-free bone. Many investigators use X-ray-photos, these are often very seductive, but cannot be used as isolated proofs of rickets or the healing of rickets, as an osteoporotic bone will appear with the same nice epiphyseal line as a normal bone. In none of the experiments have the procedures mentioned shown that a substance, which before

radiation or oxidation etc., was unable to increase the growth of the animal on a certain diet, has been able to do so after radiation etc.. On the contrary, substances which were previously able to produce growth lose this ability totally, and all the animals stop growing or even lose weight; simultaneously the consumption of food fails. And in spite of the fact that all investigators of rickets agree that rickets is only developed in the growing organism, they all forget this condition and maintain that even if the animal has been deprived of all the requirements for growth, it can be proved that a substance or an influence has acted antirachitically on their state of health.

Investigations on the Content of Vitamin in oxidated Cod-liver Oil.

As a contribution to the investigation of this subject I shall mention experiments by *L. S. Fridericia & S. Gudjónsson* (65) which from the beginning were designed to examine the relation between the ability of fresh and of oxidated cod-liver oil to act on photographic plates, and the content of A-vitamin in cod-liver oil.

As this experiment may just as well be utilized to examine whether cod-liver oil by the procedures employed loses its anti-xerophthalmic ability, but keeps the antirachitic power, I have also used this experiment for histological and chemical bone examination.

The aspects of this experiment which relate to the growth-promoting vitamin and photo-activity of the cod-liver oil have previously been described by *Fridericia & Gudjónsson*, while they did not go into the question whether any antirachitic ability of the oxidated cod-liver oil could be observed.

The control animals got our usual $\div$ A-diet. But one group of animals got a daily addition of respectively 1 mgm, 1.5 mgm, 2.0 mgm, 5 mgm, 10 mgm, 20 mgm, of freshly produced, non-oxidated cod-liver oil. This cod-liver oil had no photo-activity (i. e. the ability to influence a photographic plate) not even after 20 hours of exposure.

If cod-liver oil was exposed to a bubbling through of air, after 3—4 hours' thorough airing it was able strongly to influence a photographic plate. This ability was considerably weaker after 8—10 hours' oxidation and disappeared absolutely after 20 hours' oxidation. This oxidated cod-liver oil had lost its characteristic smell.

For the experiment was now used that sample of cod-liver oil which had obtained the highest photo-activity (4 hours of oxidation). This substance was dissolved in coco-nut oil and in certain doses given to animals arrested in growth.

From the curves it will be seen that all the animals (P 45, P 48, P 57, P 40, P 53) which got 2 mgm or less of the fresh cod-liver oil all stopped gaining in weight. They died under the symptoms of emaciation, xerophthalmia and purulent inflammations. Those animals (P 38, P 43, P 36) who received 5 mgm and upwards of fresh cod-liver oil all grew and thrived equally. The autopsy showed normal conditions. P 35 and P 54 got 0.2 gm coco-nut oil per diem and in all respects proved like animals on a pure $\div$ A-diet.

P 52 got 0.2 mgm coco-nut oil in which was dissolved 1 mgm of the cod-liver oil oxidated for 4 hours. P 42 and P 51 got 2 mgm, P 41 got 5 mgm, P 49 and P 56 and P 60 10 mgm, P 39 20 mgm, P 58 and P 59 got 50 mgm of oxidated cod-liver oil dissolved in coco-nut oil.

These animals behaved in all respects like animals on a pure $\div$ A-diet: died under symptoms of cessation of growth, emaciation, xerophthalmia and purulent inflammations.

The histological bone examination of these animals showed, in P 35 and P 54, ($\div$ A-diet with 2 gm pure coco-nut oil), considerable osteoporosis without symptoms of rickets. P 45 and P 48 as also P 57 and P 40 and P 53 showed considerable osteoporosis, no osteoid tissue, but in some cases were still found preserved cartilage cell columns in the provisional calcification zone, thus the absolute inhibition of growth in the proliferous zone was not quite pronounced. The animals with 5 mgm per diem or upwards of fresh cod-liver oil showed normal bone development.

All animals getting an addition of oxidated cod-liver oil from 1—50 mgm per diem showed cessation of growth and osteoporosis. In some of the animals were still found some preserved cartilage cells in the provisional calcification zone, but no osteoid tissue and very few and thin spongy trabeculae.

The chemical bone analyses showed quite similar conditions: The animals which got 2 mgm of fresh cod-liver oil or less, and the animals which had been on a pure $\div$ A-diet showed very hydrous bone. All animals which got an addition of oxidated cod-liver oil showed quite the same composition of the bones as animals on a pure $\div$ A-diet. The water content was just as high. Neither had the ash, calcium or phosphorus content increased, neither calculated in relation to dry fat-free nor to fresh bone. On the contrary, all the bone analyses from those animals getting 5 mgm of fresh cod-liver oil or upwards show a pronounced increase of the mineral content and a considerably reduced water content.

Therefore this experiment cannot serve as a proof that cod-liver oil can be deprived of its growth-promoting ability, but still keep the antirachitic quality, in spite of the fact that the oxidated cod-liver oil used for this experiment showed a considerable photo-activity.

On the contrary, it is seen that such an influence on cod-liver oil by which its growth-promoting ability is destroyed, has simultaneously caused a cessation of growth of the epiphyseal cartilage cells while resorption of the bone tissue occurs. This is a pronounced osteoporosis and thus cannot be regarded as a consequence of an antirachitic action, but entirely as a result of an absolute lack of growth-promoting vitamin.

Bone Analyses.

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			fat per cent	mgm
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur				
÷A-diet with an addition of fresh cod-liver oil.														
P 35	30	142	32	85	50.1	48.8	51.7	25.2	12.5	18.1	8.8	4.4	0.5	0
P 54	30	100	35	67	60.1	45.3	58.2	26.4	10.4	21.7	9.9	3.9	0.9	0

No of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent			per cent fat	mgm
	beg.	final	beg.	final			ash of fat free femur	dry fat free femur	of fresh femur	ash of fat free femur	dry fat free femur	of fresh femur		
P 45	30	68	25	37	60.9	43.3	51.8	22.4	8.7	21.4	9.2	3.6	0.5	1.0
P 48	30	78	25	44	60.7	44.9	50.4	22.6	8.8	24.6	11.0	4.3	0.4	1.0
P 57	30	116	32	75	54.4	49.2	51.5	25.4	11.2	17.5	8.6	3.8	2.8	1.5
P 40	30	116	32	96	52.7	50.4	51.3	25.9	11.6	19.5	9.8	4.4	4.8	2.0
P 38	30	142	35	135	41.3	55.6	51.1	28.4	15.7	17.2	9.6	5.1	5.7	5.0
P 43	30	114	28	122	46.2	56.3	47.6	26.8	13.9	16.0	9.0	4.7	3.1	10.0
P 36	30	142	30	142	35.1	58.3	57.7	33.6	20.7	17.0	9.9	6.0	6.7	20.0

÷-A-diet with an addition of oxidated cod-liver oil.

P 52	30	79	22	40	63.4	44.6	51.3	22.9	8.3	24.5	10.9	3.9	1.0	1.0
P 42	30	87	35	59	54.7	46.6	56.0	26.1	11.1	20.9	9.7	4.1	5.6	2.0
P 51	30	74	22	40	57.4	44.7	48.6	21.7	9.1	21.9	9.8	4.1	1.3	2.0
P 41	30	100	25	80	53.9	48.9	51.4	25.1	11.2	22.2	10.9	4.8	3.1	5.0
P 49	30	71	22	34	60.7	43.1	56.1	24.2	9.4	24.0	10.3	4.0	1.3	10.0
P 56	30	103	30	45	53.1	45.0	55.6	25.0	11.3	19.7	8.9	4.0	3.3	10.0
P 39	30	116	36	86	50.4	54.9	46.2	25.3	12.5	18.3	10.0	4.9	0.3	20.0
P 58	30	66	35	45	61.2	41.2	49.3	20.3	7.8	23.6	9.8	3.7	1.3	50.0
P 59	30	73	31	37	66.1	39.9	52.9	21.1	7.1	23.8	9.5	3.1	0.3	50.0

The Influence of Enteral and Parenteral Administration of the Fat-soluble Vitamin.

After Pappenheimer, Hess, McCann, McCollum, Steenbock and numerous others thought they had proved that the fat-soluble, growth-promoting vitamin is different from the antirachitic vitamin, this view has in great measure characterized the later investigations. There are even investigators who thought they were able to prove that the growth-promoting vitamin in cod-liver oil could exert its influence in peroral as well as in parenteral administration, while the antirachitic vitamin could only affect the organism by peroral doses.

Thus Cramer (28), Zucker & Matzner (227), P. Christou (26 a) have not been able to prove any antirachitic effect by parenteral administration of cod-liver oil. On the other hand, Katherine M. Soames (233) found that intraperitoneal injec-

tions of cod-liver oil had some, though rather a slight, effect on experimental rickets.

E. Lesné, M. Vagliano & M. Widai (152) used a diet similar to *Sherman—Pappenheimer's* diet No. 84 (gluten flour 95 per cent, calcium lactate 2.9 per cent, NaCl 2 per cent, and ferri citrate 0.1 per cent). Young rats were put on this diet, and 15 days after the commencement of the experiment were given a subcutaneous injection of 0.5 c.c. of cod-liver oil every fifth day. The animals were killed on the 35th day of the experiment after having had three injections. Other young rats got the same dose of cod-liver oil, but at the commencement of the experiment. The authors assume that the weight and growth were normal. It had risen from 30 to 65 gm, but all the animals had pronounced rickety symptoms. Hence they believe that the growth-promoting vitamin can be assimilated by the organism by peroral as well as parenteral administration. On the other hand, they think that the antirachitic vitamin can only be resorbed by peroral administration, for which reason it must be supposed that the two vitamins are not identical.

These experiments have the same essential drawbacks as those previously mentioned. A very inappropriate diet has been used with inadequate salt content, deficiency of fat and protein. The animals were only observed for 35 days, there was no control animal with the same dose of cod-liver oil administered per os. When young rats in the course of 35 days rise from 30—65 gm in weight, this is considerably less than normal growth. Furthermore the results are not based on histological and chemical bone examination.

When *Lesné & Vagliano* have found pronounced rickets in the animals receiving an injection of cod-liver oil, it will be seen that this corresponds exactly with my experiments, where the animals developed rickets when the diet contained sub-minimal amounts of cod-liver oil.

In spite of an inexpedient arrangement of the experiment, and though he only made use of macroscopical diagnostic, *Wilhelm Stepp* (249) held that a parenteral supply of vitamin A and D (cod-liver oil) is able to develop both qualities.

Subcutaneous injection of A-vitamin-containing substances may influence hemeralopia originating from A-avitaminosis; this is shown by *O. Blegvad* (13) and is strikingly apparent in xerophthalmia in children. For this purpose the unsaponifiable vitamin-containing fraction of cod-liver oil is, as a rule, used.

H. Behrendt (5) has treated rickets in children by administering such unsaponifiable fractions of cod-liver oil per os, and he has seen good effects.

No reports are at hand about such observations in the case of subcutaneous injections.

There is reason to suppose that fats, lipoids, cholesterol, and similar substances are badly and slowly resorbed, when administered to the organism by parenteral injection. Thus it is known that the fat from injections of camphor oil may still be found in the place of injection for a long time. Further, there are many examples showing that subcutaneously deposited cholesterol is nearly unresorbable and is often found surrounded by gigantic cells. These observations are also supported by the proof of *Leon Binet & J. Verne* (10) that the resorption of fat parenterally injected occurs by the fat being resorbed by emigrated leucocytes which act as phagocytes.

Therefore, when I wanted to examine the effect of fat-soluble vitamin, when this was given parenterally, it was clear to me that the vitamin-containing substance must be injected in a form as concentrated as possible, and in a dissolvent involving the smallest risk of infection and irritation. After several unsuccessful experiments with subcutaneous and intraperitoneal injection of an unsaponifiable fraction of cod-liver oil dissolved, partly in ether, partly in alcohol, partly emulsion in physiological salt water, I succeeded by dissolving the unsaponifiable fraction in olive oil.

Production of the Unsaponifiable Vitamin-Containing Fraction of Cod-liver Oil.

Our knowledge of the chemical structure of the vitamins is still very imperfect. To obtain knowledge hereof it has been

necessary to enquire into the chemical properties of the organic constellation to which they are bound, its solubility, its ability to be influenced and its possible destruction by various physical and chemical procedures. Thus it has long been known that A-vitamin could be extracted by treatment with ether from green vegetables, yolk of egg, butter, and cod-liver oil. Thus *L. S. Friederica* (64) has produced an extract very rich in A-vitamin by ether extraction of carrots.

But if it is desired to obtain a closer knowledge of the chemical structure, it is necessary to employ more radical processes. The first successful isolation experiments were conducted by *H. Steenbock, Mariana T. Sell & Mary V. Buell* (246) in 1921. They saponified cod-liver oil with 20 per cent of alcoholic KOH (600 c.c. to 300 gm of cod-liver oil) at 37° C. By treating this saponified mass with water and extracting with ether they were able to show that the vitamin-containing part is dissolved in the ether. This proved that the vitamin cannot be a fat nor an ether. Earlier *Steenbock & P. V. Boutwell* (239) had made similar saponification experiments with carrots and alfalfa.

Later *H. Steenbock, I. H. Jones & E. B. Harts* (243) have produced a non-saponifiable residuum of cod-liver oil which proved to correspond exactly in strength to the quantity of cod-liver oil from which they had made the extract. *Harry E. Dubin* (46) showed that the non-saponifiable fraction of cod-liver oil is effective against rickets in children. *Steenbock, Sell & Buell* further showed that the content of A-vitamin in butter and cod-liver oil is not dependent on the amount of certain yellow pigments of the group of carotinoids and xanthophyl. But from these experiments as well as from experiments carried out by *S. S. Zilva & M. Miura* (274, 275) and by *E. Poulsson* (196) it appears that cod-liver oil is considerably more (about 250 times more) vitamin-containing than butter.

In experiments for the purpose of isolating A-vitamin the various investigators all work with cod-liver oil and employ the method of saponification.

The investigators who seem to have gone farthest in the

isolation of the fat-soluble A-vitamin are the Japanese investigators *K. Takahashi, Z. Nakamiya, K. Kawakami & T. Kitasato* (255).

By very thorough experiments of saponification, precipitation, and extraction they have produced a highly purified substance which they call *Refined Biosterin II* and which they regard as nearly identical with biosterin, the name they propose for the fat-soluble vitamin A. They also employ saponification of cod-liver oil — 1 kilo — with 2 litres of 20 per cent alcoholic KOH. The saponification takes place in the course of 40 minutes at 40° C. and during stirring.

The saponified product is purified in 30 per cent alcoholic CaCl_2 and carefully mixed to transform the potash soaps to lime soaps. The lime soaps and most of the fatty acids are with difficulty soluble in alcohol. The lime soaps are removed by filtration. Then the soluble $\text{Ca}(\text{OH})_2$ is transformed into CaCO_3 by the alcoholic solution being blown through with CO_2 . The precipitated CaCO_3 is removed by filtration. The lipochromes are absorbed to CaCO_3 and are removed with this. The alcoholic solution is distilled at a low temperature and under vacuum under CO_2 and the residuum is treated with petroleum ether. An unsoluble mass is left over, consisting essentially of glycerine and CaCl_2 . The etheric solution still contains soluble lime soaps. The solution is thereupon washed with diluted HCl, whereby the fatty acids are liberated and transformed to potassic soaps, which are removed by washing out.

The etheric solution is dehydrated with anhydrous Na_2SO_4 and is thereupon vaporized under CO_2 and vacuum. Hereby is obtained a residuum which is reddish, half compact, about 4—5 gm from 1 kgm of cod-liver oil.

This residuum is dissolved in a small amount of alcohol on ice, on standing over large white crystals are precipitated. The form of these crystals, melting point (148° C), and other peculiarities show that it is cholesterol. The remaining solution is refrigerated, is treated with alcoholic KOH and petroleum ether and then stands over for 24 hours. In this way the glycerines are saponified and the non-saponifiable part goes into the petroleum ether. By airing of the ether the residuum is dissolved in a little 90 per cent alcohol at $\div 10^\circ \text{C}$. for several hours, whereby a further precipitation of cholesterol takes place. This process is repeated until no more precipitation of cholesterol takes place. Then 1 per cent. digitonin alcohol (90 per. cent.) is added, the mixture is heated and refrigerated. The mass precipitated from the digitonin is removed. In the liquid is now found a substance they call »crude biosterin« and which constitutes 0.1 per. cent. of cod-liver oil, and which they have shown by experimental feeding is rich in A-vitamin.

Gradually they had used 2000 kilo of cod-liver oil. A solution of 20

gm of »crude biosterin« in 2—300 c.c. of petroleum ether they treated with »animal charcoal« and short standing over. In this way the solution becomes decoloured, more golden. It is poured into a separatory, and equal parts of 90 per cent. methyl-alcohol are added. The liquid is shaken. In this way the »crude biosterin« is separated into two parts, the petroleum ether and methyl alcohol. These two are separately shaken with methyl alcohol and petroleum ether respectively, the shaking repeated several times.

The solution of petroleum ether is mixed and vaporized, there remains a residuum: yellow reddish, transparent and viscid (10—12 gm). This substance they regard as nearly pure, and call it refined biosterin II, all further attempts to purify were impossible.

They have made a determination of atoms and found: C: 81.05 per. cent., H: 11.04 per. cent., molecular weight 376. Its formula they calculate to be $C_{27}H_{44}O_2 = 400$, while that of the cholesterol is $C_{27}H_{46}O_2$, iodine figure: 185.9, specific gravity: 0.9572 (15°). Refraction index: 1.56760 (20°).

They have made similar isolation experiments with green leaves and vegetable parts.

They arrived at the result that biosterin is coloured and resembles carotinoids, but that it does not like carotinoids or lipochromes give any absorption in the visible part of the spectrum.

They find that the substance affects the photographic plate, can influence K—J amyle -paper and p- nitrophenylendiamin with colour alteration, and also reduce copper or aluminium plates. If it is dissolved in chloroform, and concentrated H_2SO_4 is added drop by drop, an indigo blue colour will appear. This reaction is positive for I: 1 000 000 molecular solution.

They find that biosterin can absorb 3 molecules of hydrogen as well as 3 molecules of oxygen and infer from this that it contains 3 double-bindings. The two atoms of oxygen are found as hydroxyl. A tertiary and a primary or secondary hydroxyl binding, which forms ether. One of the hydroxyls has the character of tertiary alcohol. They draw the conclusion that biosterin is a di-alcohol of unsaturated nature.

In the experiments on rats they find that the dose of biosterin necessary to promote growth is 0.000 001 gm per diem. They found that administration of considerably larger doses (about 10.000 times as large (1 ctgm per diem)) had a lethal effect. The animals developed alopecia, paralyses of the hind limbs, emaciation, cessation of growth and died in the course of a few hours until 7—19 days. Upon autopsy of such overdosed animals is found fat-degeneration of the liver, kidneys and heart, hæmorrhages in the digestive-canal and in the lungs.

As I did not venture with the means at my disposal to make any attempt to produce a pure A-vitamin, I limited my objects

and tried by simpler experiments of saponification to obtain a non-saponifiable fraction which, administered in small amounts, is still effective and can easily be given to the experimental animals.

For this purpose I used two methods.

I. 60 c.c. of cod-liver oil was dissolved in 300 c.c. of pure ether. 10 gm of pure metallic sodium was added to 400 c.c. of abs. alcohol by which was formed $\text{NaC}_2\text{H}_5\text{O}$. This last solution was poured into the first and the mixture stood over till the next day.

On the mass of soap thus formed was poured boiled out, distilled water, whereby the soap is dissolved and the ether partly steams away. The mixture was poured into the separatory and to it was added equal parts of pure ether and it stood over for 24 hours. The mixture separated into a lower, madeira-coloured, soap-containing layer and a higher pale yellow layer (the ether layer). The lowest layer was tapped, and the highest was mixed with equal parts of boiled out, distilled water, and was shaken. Repeated washing out of the ether. Thereupon vaporizing of the ether under vacuum (about 60—100 mm pressure). This leaves a half compact, brownish clear mass, about $\frac{1}{2}$ gm.

A small amount of this was dissolved in petroleum ether and was examined after the method elaborated by *N. Sjørøsløv* (232) by the addition of 1 drop of concentrated H_2SO_4 whereby the solution turns distinctly violet.

II. The second method I used was an attempt to follow the method given by *K. Takahashi* and Co-workers. I followed this to the stage of precipitation of the cholesterol at $\div 10^\circ \text{C}$. and also here succeeded in getting a brownish residuum, which gave H_2SO_4 reaction.

I had previously examined biologically the non-saponifiable fraction produced after the first-mentioned method. I used half of the fraction from 60 c.c. of cod-liver oil, dissolving it in pure olive oil and making this enter as 15 per cent. of fat into our customary basal diet. As will be seen from the curves for R 30 to R 39, R 33, R 34, and R 35 grew quite as well as R 36, R 38, and R 39 which had 15 per cent. of butter fat added to the diet. The calcification degree and histological formation of the bones were alike for these two groups and the bones were in every respect normal (see the table p. 70).

I had earlier tried to inject partly an etheric solution of the

non-saponifiable fraction, partly an alcoholic solution, partly an emulsion in physiological NaCl solution, but I never obtained growth of the young rats in this way. Necroses at the places of injection and inflammations occurred.

As known, an emulsion of the substances like cholesterol can easily be made with a hydrous solution of bilious acid salts. But as it is also known that bilious acid salts, when deposited subcutaneously or intraperitoneally may develop irritation, I desisted herefrom and dissolved the non-saponifiable fractions I and II separately in 10 c.c. of olive oil.

In my experiment I have not endeavoured to measure the degree of the effect of the non-saponifiable fractions, I have only tried to prove that parenterally injected A-vitamin can produce precisely the same effects as peroral administration, that is to say cause absolutely normal growth and bone formation in animals kept on pure $\div$ -A-diet.

I used 8 young rats of the same litter 47 days old. The animals were put on $\div$ -A-diet. R 122 at the commencement of the experiment got a subcutaneous injection of 0.4 c.c. of I. R 123 the same dose intraperitoneally.

R 124 and R 125 were given respectively subcutaneous and intraperitoneal injections of 0.5 c.c. of II.

These animals rose rapidly in weight during the first 4—5 weeks, but then the rise of weight stopped and the animals began to lose weight. Symptoms of xerosis conjunctivae occurred. Then injection was given again of the preserved solutions of non-saponifiable fractions in olive oil. But as these had evidently lost their efficacy, I produced a new portion (according to method I), which was then injected into R 122 and R 123 and these then rapidly rose in weight in the following weeks. The xerophthalmic symptoms absolutely disappeared.

R 124 and R 125 which were not given fresh injections, died under the symptoms of A-avitaminosis and furthermore developed peritonitis on account of the injection.

After 5 weeks on A-free diet R 126 was given an injection of II, but died the following day from purulent inflammations. R 127, after 7 weeks on A-free diet had 1 c.c. of II intraperitoneally injected, but died 14 days later with the symptoms of A-

avitaminosis and peritonitis, no visible oil accumulation was found at the places of injection, but the tissue was more or less characterized by inflammation and edematous. As already mentioned, there is reason to suppose that the resorption of parenterally administered fat takes place very slowly and occurs principally by phagocytosis.

R 128 and R 129 were the whole time kept on $\div$ -A-diet. Only R 122 and R 123 of these animals showed normal bone development and normal bone growth, while the bones of all the other animals showed cessation of growth and osteoporosis, though considerably less pronounced in the case of R 124 and R 125. These two animals had also a considerably greater content of minerals in the bones than R 128 and R 129, which were kept the whole time on $\div$ -A-diet without injections.

From this experiment it appears that A-vitamin administered parenterally is in all respects able to replace the peroral supply.

Animals on a pure A-free diet, but receiving subcutaneous or intraperitoneal injections of the non-saponifiable fraction of cod-liver oil are prevented from getting xerophthalmia. Their bone development is normal both histologically and chemically.

But the thus injected A-vitamin can only influence the organism as long as there is still found a depot of it in the place of injection, as soon as all is resorbed all the symptoms of A-avitaminosis occur rapidly. These can again be brought to disappear by a renewed injection.

The resorption of parenterally administered fats and lipoids takes place slowly.

Bone Analyses.

No. of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent		P per cent		per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur	ash of dry fat free femur	of fresh femur	

$\div$ -A-diet with parenteral administration of A-vitamin.

(R 122 and R 123 had 2 injections, R 124 and R 125 one injection).

R 122	47	115	46	120	45.3	55.4	53.0	29.4	15.7	16.6	9.2	4.9	2.5
R 123	47	115	49	136	49.0	53.0	54.3	28.8	14.2	16.3	8.7	4.3	3.1
R 124	47	98	46	78	53.2	50.8	59.2	30.1	13.0	19.3	9.8	4.2	7.5

No. of rat	Age in days at the		weight at the		H ₂ O per cent	ash per cent of dry fat free femur	CaO per cent			P per cent				per cent fat
	beg.	final	beg.	final			ash of dry fat free femur	of fresh femur		ash of dry fat free femur	of fresh femur			
R 125	47	98	40	70	53.6	59.8	59.6	30.4	12.0	17.9	9.1	3.8	8.8	
R 126	47	72	45	60	54.0	53.3	53.0	22.9	9.8	21.7	9.4	4.0	6.6	
R 127	47	112	51	67	58.0	45.1	52.7	23.7	9.9	20.2	9.2	3.8	0.2	
R 128	47	98	47	54	56.0	46.5	52.4	24.3	10.1	22.3	10.3	4.3	5.8	
R 129	47	98	39	50	56.5	42.9	51.8	22.2	9.6	19.5	8.3	3.6	0.2	

III.

THE PATHOGENY OF A-AVITAMINOSIS.

As I have often repeated in the preceding part, animals on an A-vitamin-free diet show cessation of the growth and weight curves in the course of 4—5 weeks, the eye borders get hairless, the fur thin and bristling. Enophthalmus appears, and a mucous secretion, often bloody, from conjunctivae and cornea. The eye-injuries may be further developed to keratomalacia, hypopyon, panophthalmia, anophthalmia. The bones show osteoporosis and absolutely arrested growth in the proliferous zone. But further otitis and mastoiditis purulenta (*Daniels & Armstrong* (31), *Howard, McCordock & Corogdon* (119)), abscess of the root of the tongue, pneumonia, cystopyelonephritis, inflammation of prostata and vesiculae seminales are rather commonly found.

S. Mori has proved that a very wide-spread atrophy in most of the glands in the organism is found in A-avitaminosis. Thus he has described xerotic changes of the epithelium in gland: submaxillaris, sublingualis, parathyroid and in *Meibom's* glands, in *Harder's* gland as well as in the epithelium in larynx and trachea. He therefore thinks that the symptoms in all these organs in A-avitaminosis are a result of hypofunction and atrophy of these glands.

E. Stransky (252) has reported 6 cases of keratomalacia in children and believes that only one of them can be regarded as A-avitaminosis, and this was cured by cod-liver oil therapy.

The others died from pyodermia, acute dyspepsia, pneumonia and one from stenosis pylori. They had all of them for a long time shown cessation of growth and loss of weight, that is to say, the very symptoms of arrested growth (A-avitaminosis!).

A. M. Yudkin & R. A. Lambert (272) on the contrary maintain that the symptoms of inflammation which are often found in these organs are the primary ones in the picture of the disease, and that the xerotic and keratinising phenomena in the epithelium are a consequence of the inflammation.

S. Wohlbach & P. R. Hove (276) are, however, the investigators who have made the latest and most extensive investigations of these problems.

They made their experiments on albino-rats receiving the A-free diet of *Osborne & Mendel* (Casein, 18, starch, 51, lard 24, beer yeast 3, salt mixture (*McCollum* No. 185, same as used by me)). The control animals had butter fat instead of lard.

Like earlier investigators they found that the macroscopical changes in the organism of A-free animals consist of emaciation, atrophy of liver and spleen, often also of the glands: submaxillaris, parotis, lacrimalis, *Harder's* gland, furthermore of pancreas, thyreoidea, hypophysis, thymus and testes. The kidneys, parathyroids and suprarenal glands were not found to be atrophic.

The liver atrophy they try to explain by the decrease of fat and glycogen. The atrophy of the spleen by the emigration of lymphocytes. On the other hand they occasionally found that the specific secreting gland-tissue in submaxillaris and *Harder's* gland may have disappeared. The reason for this they seek in a cystic transformation of the gland by the excretory ducts being filled up with desquamated and retained epithelium-cells, the epithelium is keratinized.

This keratinization they also find in the respiratory tract (nares, the nasal cavities, larynx, trachea, bronchiae) further in the alimentary tract: salivary glands, the glands of the tongue, pharynx, pancreas duct, in the ducts of the urinary tracts, vesica, ureter, pelvis, in the ducts of the genital organs, in conjunctivae and cornea and the glands of the eyes, furthermore in the hair follicles and sebaceae glands.

The changes can be proved earliest in the excretory ducts, later in the acinus of the gland.

They found that injection and suppurative inflammations were very common accompanying phenomena, but that they

were not always present, and do not seem to be necessary for the occurrence of the keratinizing processes.

They have only undertaken few investigations for the mitochondria of the epithelium and could not thereby prove any real deviation from normal facts in A-avitaminosis. They find that the cytoplasm is diminished, but that the nucleus has not altered in size.

In advanced cases they also find complete arrest of the growth in the epiphyseal cartilage and in the bones, but no symptoms of osteomalacia, rickets or scorbut. In the bones were shown similar phenomena by *Hess, McCann & Pappenheimer* (91) when feeding with A-free diet.

Hove found similar processes of keratinization in several epithelia and glands in a child that had died from A-avitaminosis.

The explanation which *Wohlbach & Hove* give of their numerous observations seems less obvious. They mention that they find numerous mitoses in the epithelium and take this as a sign of rapid and increased growth.

This seems to be absolutely at variance with the description of atrophy in the secreting epithelium of the glands. Nor does it appear from the micro-photos that particularly numerous mitoses are found, this might more easily be taken as a sign of slow growth, a protracted dividing of the cell, that the cell has perhaps remained in the stage of mitosis and cannot be developed further on account of inhibited growth. When then moreover the special gland epithelium has partly or entirely ceased to secrete, the continued current is lacking which under normal circumstances would carry off the desquamated cells of the excretory ducts. In these ducts there may in this way appear such a picture of the cylinder epithelium as may simulate stratified epithelium, and these accumulated dead epithelium cells will then lose their cell-limits and the nuclei their staining ability and partly be fused together into corpuscles like keratine. Further there is reason to believe that the growth under all circumstances is somewhat more rapid in the excretory ducts than in the acini, which also contributes to the accumulation of the

epithelium cells in the excretory ducts, and the obstruction of these, and thereby to the formation of retention-cysts in the acini.

Starting from these considerations, I took various glands, eyes and other organs from the experimental animals already in the early series of my experiments to examine the changes which may have occurred in animals on an A-free diet.

While in most of the cases the organs showed distinct symptoms of infection and purulent inflammations, I also succeeded in finding organs without symptoms of inflammation, thus in these cases the change cannot be considered a consequence of infection judging from the histological examination. Bacteriological examination was not made.

I found in eyes, salivary glands and bronchi changes exactly corresponding to the descriptions of *Wohlbach & Hove*, not numerous mitoses, however, but on the other hand distinct atrophy of the gland acini, low cylindric, flat epithelial cells in the excretory ducts, desquamation of these cells and accumulation of the same, often in such a way that they fill up the lumen, and block this, which causes retention cysts of the gland. In several places is seen »keratinization« of the cells, these have become clear, refractive without marked cell-limits and with no or only slightly stained nucleus, in many places is formed a distinct stratified epithelium, which in some places is horny.

As regards the cartilage bone limit, I have already previously described the cessation of growth in the cartilage. Resting non-differentiated hyaline cartilage cells are found right into the marrow cavity, in many of the bones, however, there is still found a certain column-formation of cartilage cells in the region corresponding to the proliferation zone, but these columns are short, thin, and terminate suddenly, they show no enlargement of the cartilage capsule, nor retraction of the cartilage cells nor the presence of several cartilage cells in the same capsule, nor is there any calcium deposition in the cartilage matrix.

As another essential symptom of the universal retardation of growth, supposed by me, I shall finally mention the bone marrow in these animals on A-free diet. While in the animals showing preserved, though some reduced, growth, and simultaneously

rachitis florida, I cannot prove real changes in the bone marrow, apart from the irregularity and the enlargement of the provisional calcification zone and the osteoid tissue, I find that animals arrested in growth on A-free diet show a picture of the bone marrow differing from that of a normal animal.

The marrow cavity is found to be enlarged as a consequence of the osteoporosis and the bone resorption. The fat is reduced and the cavity filled up with dilated blood vessels. The marrow tissue is atrophic and hypoplastic. Instead of the very polymorphous picture of normal marrow tissue with myelocytes, erythroblasts, normoblasts, erythrocytes, eosinophilous cells, mast-cells and numerous megakaryocytes, a somewhat less polymorphous picture is found in the bone marrow of an A-free animal. Often greater or smaller heaps of small cells with small protoplasm border and dark nucleus are seen, these resemble monocytes (lymphocytes), furthermore the myelocytes are fewer in number and smaller than normal ones. The erythroblasts also are small. There are found considerably fewer megakaryocytes in every field of view, and the nucleus is less lobated.

Thus also in the bone marrow distinct symptoms of cessation of growth are found. This will surely also explain the severe anaemia which often accompanies rickets.

Further these facts will perhaps explain the reduction of the number of blood platelets which *Drew & Mottram* (29) proved can be found in animals with A-avitaminosis. Such a reduction has also been made probable by the investigations of *Johs. V. Andersen* (1) on A-free rats.

Whether the blood platelets, as mentioned by *Wright*, but disproved by *S. Petri* (191), are formed of megakaryocytes or are formed of erythrocytes by sequestration (*Maximow & Schwalbe*) or of the nuclei of the erythrocytes (*Pappenheim*), they are by all investigators supposed to be formed in the bone marrow. If now the growth here has ceased, it must be supposed that the formation of new blood platelets and erythrocytes stops.

IV.

CONCLUSION.

If now I am to sum up what I have elucidated by my experiments and believe I have rendered probable I will characterize the influence of A-avitaminosis on the organism as a cessation of growth.

When young rats are fed on pure A-free, otherwise adequate diet, they will for the first 3—4 weeks consume normal amounts of food, 6—10 gm per diem, but then they lose their appetite somewhat and now only consume 3—5 gm daily. This is, however, considerably above the rather pronounced starvation met with in animals on B-vitamin-free diet. The rise of weight stops in 4th—5th week, it is to be supposed that the growth energy of the cells is now extinguished as a consequence of the deficient supply of A-vitamin and total consumption of the stored up deposit of the organism.

Simultaneously with the arrest of the weight the other symptoms of cessation of growth occur: the eye borders get hairless and the fur bristling and thin as a consequence of arrested growth in the hair follicles and sebaceae glands. Secretion from conjunctivae and xerosis conjunctivae et cornea appears. The cessation of growth in the epithelial cells in cornea and conjunctivae is also here the causal factor.

No formation of new cells on lamina elastica anterior takes place, the above lying cells are not sequestrated with the usual speed, this will cause the farthest cells to die, keratinize. As the normal and constant epithelial growth with the consequent sequestration of the upper cells must just be supposed to be a

considerable protection against the invasion of bacteria and against the occurrence of inflammation, this will easily commence when the normal renewal of the epithelium has ceased or is inhibited. In this way inflammation phenomena become an epiphenomenon to the A-avitaminosis. In a similar way the inflammations in all the other organs, which occur with A-avitaminosis may be explained.

To this is further added the atrophy and hypoplasia of the secreting epithelia as well as a universal regressive metaplasia of the epithelial cells in numerous organs.

In the bones the absolute retardation of growth will cause cessation of the proliferation of the cartilage cells, and as it is the function of the normally proliferating cartilage cells to assimilate calcium and deposit this in the cartilage matrix, thus the assimilation of calcium will also have ceased. The bone marrow cells do not grow any more, do not divide, and therefore do not form erythrocytes and blood platelets, therefore anaemia and a reduced number of blood platelets are found.

When now small amounts of A-vitamin are added to the diet of such control-animals, a condition will be reached, where the administered amount of A-vitamin just proves to be sufficient to keep up some cell growth. This is proved by the non-appearance of the xerophthalmia. The animals keep their weight or a slow and slight rise of weight takes place. In these animals there cannot be found marked signs of arrested division of cells (arrested growth!) in the organs, but it will be found that the growth of the bones does not go on with normal rapidity. The cartilage cells have only the growth impulse in slight degree, the dissolution of these during transformation of cartilage cells and osteoblasts to bone cells is retarded. This causes the deficient calcification in the provisional calcification zone, by which the osteoid tissue arises. The differentiation of the cartilage cells is retarded or stopped. Therefore they remain as irregular cartilage cell columns or isles in the marrow cavity.

With a greater content of A-vitamin in the diet the growth of the cartilage cells will take place more rapidly, they will more rapidly and to a higher extent deposit calcium in the

cartilage matrix and likewise the newformed spongy trabeculae will by the activity of the osteoblasts more rapidly and more completely resorb the calcium.

When at last the proportion is reached when the vitamin content of the diet is sufficient for normal growth of the animal, the bone formation will also show normal conditions and show no rickety symptoms any more.

But as will be seen from all the experiments with butter, there is rather a long step from the addition of A-vitamin which is sufficient just to preserve growth, to the addition required for the growth to be in all respects normal, and it is just between these two extremes that the amounts of A-vitamin are found which cause rickets to develop.

Thus we are also able to explain the numerous experiments of the retention of calcium in the normal and in the rachitic organism on different diets.

As previously mentioned, there is often in breast-fed children found a calcium retention of up to 70 per cent, while most of the investigators, by feeding with cow's milk, find only 40 per cent or even as little as 16 per cent.

Normal mothers may be supposed to yield an abundantly vitamin-rich milk, while as regards cow's milk it has been shown that it may be rather poor in vitamin, especially during the winter, on account of the vitamin-poor food the cow gets.

Further it may be supposed that the resorption of calcium and other salts from the intestinal epithelium may in itself take place in the same way and to the same extent in normal and in rickety children. But while the first, who grow and grow normally are in need of much calcium for the increased mass of the organ and especially to satisfy the calcium avidity of the normal, growing cartilage cells and bone tissues, the growth-inhibited organism, and the organism in which the differentiation of cartilage cells occurs more slowly, will not be able to deposit calcium, for which reason this is again excreted in the intestines. Thence the slight calcium retention in rickety individuals. But thence also the sparse calcium retention on a diet poor in A-

vitamin (cow's milk, especially the winter milk, carbohydrate food etc.).

Thus *Olaf Bergeim* (7) found in experimental rickets that the Ca-resorption in the upper part of the intestinal canal is normal, but that the excretion of calcium in colon is again increased by rickets.

The more rich a diet is in A-vitamin, the greater the retention of calcium will be, just because of the growth, within certain limits, being increased proportionally with the content of A-vitamin in the diet.

Now we can better understand why rachitis appears especially during the winter and spring months. In these months the cow's milk is rather poor in vitamin, while the calcium content of the milk must be supposed to have been relatively constant. The rise in weight and the growth of the child is therefore smaller during winter, and its Ca-retention is reduced, therefore rickets is developed. This rachitis florida may become of a more hyperplastic form, if you succeed in starting growth again by increased but still suboptimal supply of A-vitamin. It is proved by *H. Steenbock, E. B. Hart, C. A. Elvehjem & S. W. F. Kletzien* (242) that for quick-growing animals such as chickens and kidlings a considerably greater content of vitamin in the diet is required before changes in the calcium metabolism can be proved.

That breast-fed children may also develop rickets is explained by the fact that the mother-milk during winter and spring may also be poor in vitamin. This again must be attributed to the scanty content of vitamin in the diet of the mother. Precisely in the Northern and temperate countries the diet of the poorer class in the country and in the cities is only little vitamin-containing during the winter and spring months. Fresh vegetables, eggs, milk and butter enter only in small amounts into the diet and the content of the vitamin in these is again dependent on the food of the animals.

In the Southern and warm countries the quality of the food stuffs will be very homogeneous all the year round. Partly fresh vegetables will be found all the year round, partly the chickens,

cows, and goats are out the whole year and thus get fresh natural food as against the artificial and dry food in the temperate zone.

Investigators have even gone so far as to assert that human milk does not contain any substance which can prevent the incidence of rickets (*E. Lesné & Vagliano* (153)) though they have not attempted to give an explanation of why rickets is so much rarer in breast-fed children than in artificially fed children.

As mentioned, *A. F. Hess*, *M. Weinstock & E. Sherman* (102) and *A. F. Hess* (82) have thought they were able to prove that ultra-violet light-baths of the mother or nurse causes the milk to become highly antirachitic, just as *H. Steenbock*, *E. B. Hart*, *C. A. Hoppert & A. Black* have shown it with goat milk, but nothing is said about the growth of the young, and the investigators all employ the inexpedient arrangement of the experiments which we previously mentioned.

There is thus no need to resort to the supposition that the absence of sunlight has a direct influence on the incidence of rickets or that the increasing intensity of the sunlight during the early part of the summer acts directly healing on rickets.

Partly we know far too little about the influence of the light on the organism, especially ultra-violet light. Partly there is not yet one experiment on animals which proves with certainty that radiation is able to replace the A-vitamin in the diet, the animals do not grow any better if radiated when they are kept on an A-free diet. On the contrary, these animals seem to use up the possible store of A-vitamin in their organism quicker than if they were not radiated.

From the few observations of and investigations on the effect of light on the organism, an augmentative effect on the metabolism seems to be in question (*C. Sonne* (234)) experiments on meal-worm pupae).

Therefore the A-vitamin store of these animals will be resorbed sooner, a transitory rise of weight in animals kept on an A-free diet may perhaps occur, but at the same time the resorption processes of the organism will take place more rapidly. The non-differentiated cartilage cells in the provisional

calcification zone will be quickly resorbed, and this cannot by any means be taken as a healing of the produced rickets, but will give place to a pronounced osteoporosis; and this, too, provides the explanation of the assumed healing of rickets by starvation asserted by American investigators. That these investigators have observed and described deposition of calcium in the previously calcium-free cartilage proliferation zone when the animal is starving or is exposed to light, may partly be explained by the fact that the cartilage matrix perhaps has beforehand contained calcium, which appears now when the cartilage cells are resorbed, partly it is possible that by the resorption processes during the consuming influence of the radiation or starvation so much phosphate is liberated that the cartilage matrix may now resorb the calcium phosphate.

L. E. Holt jr., V. K. la Mer & H. B. Chown (115, 117) have just proved that, even if the cartilage proliferation zone in rickets (on *McCollum's* diet No. 3143) seems during the first weeks to be calcium-free, at a little later stage deposition of calcium in the cartilage matrix is undoubtedly found, in spite of continued inadequate feeding.

Even though *A. Hartmann* (86) and *P. G. Shipley, I. Howland & B. Kramer* (120) have shown that »rickety cartilage« has kept its ability to bind calcium in the matrix when imbedded in rabbit-peritoneum or in normal serum in vitro it must be maintained that these conditions do not correspond to real facts in vivo, and that the cartilage in these experiments is precisely exposed to A-vitamin-containing media.

As known, the calcium content of the blood in rickets is normal, corresponding to the fact that the calcium retention from the intestines is probably normal, and to the calcium being an organic component of such importance to life, bound in albumin molecules. On the other hand the blood-phosphorus is reduced in rachitis florida. This must be supposed to be caused by the inhibited or arrested growth. If the cells of the organism do not grow, the amount of phosphate of the blood cannot be deposited in the tissues, especially as no processes of proliferation are found in the cartilage and bone tissue, and there being

thus a reduced demand for the content of phosphate in the blood, the blood will be less charged with phosphorus, and the phosphorus which has possibly been resorbed in the upper part of the intestinal canal will soon be excreted in colon.

But as soon as either by starvation or radiation a greater demolition of cells in the organism, a greater resorption of own tissue, takes place, then the blood will again »endogenously« be charged with phosphorus and therefore the blood-P' will under these conditions be found increasing from the abnormally low site.

The process is reversed in rickets which is healed by a supply of A-vitamin. Here it will be the resumed growth, especially of the cells of the cartilage and bone, which makes increased demand on the phosphate content of the blood and less phosphate is excreted in colon, as the phosphate is retained in the growing bone tissue. Therefore a real increase of the content of phosphorus in the organism takes place »exogenously«.

The more intimate and detailed influence of A-vitamin, how it acts upon the cells of the organism or enters into the metabolism of the cells, we cannot explain with our present knowledge.

How A-vitamin causes growth of the cells, division of the cells, secretory activity, in short, cell-function, we know nothing about. That it is hardly a question of any function, as amboceptor or katalysator etc. but that it really enters into the metabolism seems to be made probable by the fact that 1) a certain not inconsiderable supply of A-vitamin is required to maintain growth and 2) a still greater supply to make the growth normal in all respects.

3) As soon as the organism has used up its own scanty reserves, and a new supply of A-vitamin does not take place, cessation of growth with all its symptoms occurs.

4) If the organism is supplied with such amounts of A-vitamin that a certain growth is guaranteed, though smaller amounts than are required for normal growth, then the subnormal growth will manifest itself in rickets.

5) If the diet besides having an insufficient content of A-

vitamin is also insufficient in calcium or phosphorus, the water-content of the osseous system will be further increased, the mineral content further decreased and the histological picture may, on account of the more inhibited differentiation of the cartilage cells, show a considerably enlarged proliferation zone, but comparatively small amounts of osteoid tissue.

V.

OF WHAT IMPORTANCE ARE THE RESULTS GAINED FOR THE THERAPY AND PROPHYLAXIS OF THE A- AVITAMINOSIS AND ESPECIALLY OF RICKETS IN CHILDREN?

As regards the prophylaxis of A-avitaminosis in humans the efforts should be directed towards obtaining as great a content of A-vitamin as possible in the ordinary and cheapest food articles.

To achieve this it will in the first instance be of great importance that that part of the population which is occupied with the rearing of cattle and poultry is instructed about the value of vegetables and natural food to these animals. This should result in these products being produced in a more homogeneous and more vitamin-rich quality.

That the composition of the food has great importance for the vitamin content of the milk appears from the experiments on rats of *Korenchevsky* (140) and *V. Korenchevsky & Carr* (145). These show that a vitamin-rich feeding of the mother during pregnancy and lactation plays an important rôle for the growth of the young and for the resistance of the young on a subsequent insufficient diet. But as a matter of course it does not give any continued resistance. This also appears from the experiments on rats conducted by *Hess, Weinstock & Tolstoi* (104), *Hess & Weinstock* (98), and from the observations of children by *Hess & Weinstock* (99) whose mothers had had cod-liver oil during the last months of pregnancy.

If the mother is under-nourished, she seems to use up the

supplied A-vitamin herself, and therefore her milk, in spite of doses of cod-liver oil, hardly gets very A-vitamin-containing. *E. M. Hume* and *Hess & Weinstock* do not believe that the anti-rachitic qualities of cod-liver oil can be transferred to the children through the mother milk.

The experiments of Drummond have proved that feeding of milk cows with cod-liver oil does increase the vitamin content of the milk judging by growth experiments on rats. Similar experiments have been conducted by *R. Wagner & H. Wimberger* (263), who fed two cows during respectively 17 and 6 months with 50–300 gm of cod-liver oil daily and compared the effect of their milk with the effect of milk from cows without an addition of cod-liver oil to the diet. While in experiments on rickety children they found that the milk from cows treated with cod-liver oil was somewhat effective, *E. M. Luce* could not prove any essentially greater effect of this milk than of the control milk, judging from growth experiments on rats (the A-free diet of *Hopkins*) and from experiments on the rachitogenic diet No. 3143 of *McCollum*. To this it must be remarked that the milk samples had unfortunately been brought to London in a pasteurized state and were not tried biologically until there, further the supply of cod-liver oil to the cows had ceased in October 1923, and the effect of the milk was not examined until the winter, in children who came in then. Furthermore, both batches of cows seem to have received a rather vitamin-rich food. *E. M. Luce* only mentions the ash content of rat's bone in relation to dry fat-free bone, which may easily lead to false conclusions. Similar objections may be made to the experiments of *R. Adams Dutcher, Mattie Creighton & Henry Rothrock* (47) on the influence of rachitogenic, but radiated diet.

Therefore all mothers ought, both during pregnancy and lactation, to try to live on a diet rich in vitamin. This includes milk, vegetables, and eggs. The mother ought to learn that pasteurized milk is unfitted for nourishment for children, as too many examples are known of pasteurized milk being absolutely deprived of its content of vitamin by being re-heated when the food for the child is prepared, thus examples of xerophthalmia as a consequence of this diet have been seen.

A conventional employment of cod-liver oil for infants, especially during winter, has been tried in several places, and apparently with good prophylactic effect.

But as rachitis florida is seen in such children, too, (*L. R. de Buys & L. v. Meysenbug* (22)), this measures is rather problematic. Partly there can be a rather great difference in the content of vitamin of the cod-liver oil, for which reason this ought to be standardised, partly there is the possibility that a supernormal supply of vitamin by cod-liver oil may promote intensified growth, so rapidly that the content of calcium and phosphorus in the diet is perhaps insufficient to cover the demand of the new-formed bone tissue for calcium phosphate, and so an insufficiently calcified matrix and osteoid tissue may arise.

It must be remembered that the calcium content of the milk may be rather low. *L. R. de Buys & L. v. Meysenbug* (23) have thus found that the milk from women with healthy children is more calcium-containing (32.6 mgm CaO per 100 c.c.) than the milk from women with rachitic children (27.5 mgm per 100 c.c.) and that the calcium content decreased with the continuance of the lactation (the age of the child).

E. B. Hart, H. Steenbock, C. A. Elvehjem & H. Scott (85) observed a difference in the calcium balance in cows in the different seasons and think that this cannot be explained by the different content of vitamin in the food. And *A. Blanchetière* (11) has proved that cow's milk contains less calcium during summer than during winter, but he supposes that the greater content of vitamin in the cow's food during summer causes a greater deposition of calcium in the organism of the animal, for which reason the calcium content of the milk does not increase. This also explains perhaps why cow's milk has a lower content of phosphorus during summer, as found by *Einar Lenstrup* (150).

Ethel M. Luce (157) maintains that even if the diet of the mother animal has been very rich in vitamin, it will only be able to yield some protection against the influence of an insufficient diet to the young ones, if the original diet also has a surplus of calcium.

Therefore this relationship of equilibrium between the vitamin content and the mineral content of the diet is in itself very difficult. Add to this that there is a great difference in the children. It is a well-known observation, both as regards humans, animals, and plants that, even if the conditions and circumstances seem in all respects alike, the growth-energy and growth-ability of each individual are different. Some children grow more rapidly and get taller than others. To this must further be added that the food is differently utilized in the digestive canal. If plenty of calcium soaps are formed in the intestines, then an increased supply of fats will only aggravate this phenomenon, and diminish the resorption of fats as well as salts.

It is also from this consideration that I believe that the treatment instituted by *Czerny*, *Jundell*, and *Monrad* with low content of calories in the diet (60—70 calories per kilo per diem) is justified. In this way the utilisation of the diet in the intestinal canal is taken care of daily, the organism is kept on so slight and slow a rise of weight that the growing tissue, especially the bone tissue, even if produced at a slower rate, will nevertheless on account of the equilibrium between the vitamin content of the food and the limit for the achievable resorption of calcium and phosphorus from the diet be saturated therewith.

As a matter of course this applies only to children under medical control.

J. Jundell seems most frequently to combine his partial starvation with a supply of cod-liver oil, and often also phosphorus as well as cream, milk, eggs, and vegetables.

That it seems clinically proved that radiation with light improves conditions and apparently heals rickets must partly be explained by the fact that the diet, when the therapy of rickets commences, will always be better and more suitable than the diet under which rickets was developed. The children are often treated in hospitals, get fresher and better milk, the utilisation of the diet is controlled, the content of calories perhaps reduced, by which the growth becomes slower and resorption of

the non-differentiated cartilage cells and the osteoid tissue takes place. But also the radiation seems to increase the metabolism, by which these processes of resorption are accelerated, while simultaneously the augmented metabolism creates equilibrium between the supply of calories and the combustion, equilibrium between the demand of the growing and the differentiated cells for inorganic components, and the available supply thereof.

For this reason alone mothers should be advised to keep the children an ample time in fresh air and light.

As certain vegetables (carrots, spinach, brussels sprouts, salad) are rather rich in vitamin, these food-stuffs ought to enter into the diet of the child as soon as its age permits (about 6 months). The vegetables further offer the advantage that they are rather rich in vitamins and relatively poor in calories, while the vitamin-poor cereals supply the child with a great amount of calories and rather few mineral components.

Treatment with saline baths, not cold but of low temperature, will also, on account of the cooling and the hyperaemia in the skin, produce increased metabolism and will thus, when the food is kept sufficient and rich in A-vitamin, contribute to establish this equilibrium.

From all these facts it will be seen how difficult it may be, not only for the mother but also for the physician, to help the child through the first one or two years of its life. These difficulties are even greater for the child since its life and physical condition are dependent on the difficulties being removed in the right manner, so that the child will not later in life have to bear the burden of rachitic deformations.

As the child grows older these difficulties will fortunately grow less for all parties, as its nourishment is less threatened by the deficiencies mentioned, and its growth has entered into a less rapid phase.

VI.

SUMMARY.

1) A thorough description of the normal bone development and bone growth is given. On this background the pathologico-anatomical picture of rickets is described. Report and criticism of the existing theories about the aetiology of rickets.

2) The two hitherto sole-prevalent forms of diet (*McCollum's* No. 3143 and *Sherman—Pappenheimer's* No. 84) which have been utilized for studies on experimental rickets in rats are criticized as being unsuitable and inexpedient. They are not as synthetically composed as it is possible, and they are insufficient in respect to too many factors simultaneously (protein, fat, A-vitamin (which is variable), B-vitamin, and numeral mineral substances). These forms of diet will thus not be able to furnish any information about the essential aetiological factor.

3) The period of experiment on this regime is always of too short a duration to provide certain information especially about the histology and the composition of the bones.

4) The period of experiment chosen is furthermore unfortunate, as young rats, when they are put on such an insufficient diet about 30 days old, are often able to grow as much during the following 3—5 weeks as young rats on an adequate diet. Thus we cannot form a reliable estimate of the weight and growth of the animals.

5) The investigation methods employed give rise to false conclusions. The macroscopical inspection of the skeleton cannot show decisively whether normal bones or osteoporotic bones are before us. It is shown that osteoporosis is found when the experi-

mental animals have stopped growing or lost weight, and that the pathologico-anatomical picture in such cases is mostly described as normal by earlier investigators.

6) »The line test« must be considered as unfitted for distinguishing between an osteoporotic and a normal or healed bone, since calcium precipitation has also been proved in the cartilaginous proliferation zone when the animal has for some time been kept on the rachitogenic diet without any therapeutic measure being taken.

7) Röntgenograms may show whether the cartilagenous proliferation zone has become narrower, and may perhaps reveal whether calcium precipitation has occurred, but röntgenograms cannot show whether the bone as a whole has become richer in minerals and poorer in water, that is to say, they cannot show whether the bone has become more normal or more osteoporotic.

8) The estimation of the content of calcium and phosphorus in the blood cannot as yet be used as a diagnosticon for rickets or the healing of rickets, for this purpose the content is too much affected by uncontrollable influences: the duration of the experiment, starvation, the influence of light, changes in the salt content of the diet, supply of cod-liver oil etc.

9) In most of the earlier experiments two of the most important and reliable methods of investigation are left out, viz. the histological bone examination, and the chemical bone examination. The histological bone examination may show whether we are concerned with a normal bone with normal growth or with a rachitic bone with some, but retarded and incompletely differentiated growth, or finally with an osteoporotic bone, with rarefaction, lacunar bone resorption, and arrested growth in the cartilage.

The chemical bone examination will give information about the water content and ash content of the bone. This method of investigation is as essential as the histological method, as only this is able to give information as to whether a previously rachitic bone has by some therapeutic measure become poorer in water and richer in minerals (i. e. healed), or whether the water content has increased and the mineral content decreased (i. e.

the bone has become more osteoporotic). It here depends on the ash content compared with that of the fresh bone and not with that of dry fat-free bone. A very osteoporotic bone may be so hydrous and contain so little organic substance that the percentage ash content seems high when compared with dry fat-free bone, while calculated in relation to the fresh bone it is in reality very low.

10) It is thus shown that erroneous conclusions have probably been drawn in numerous of the earlier experiments on these rachitogenic regimes, and that, on account of the inhibitory nature of the regimes, it has not at any rate been possible to give a decisive answer to the questions it was proposed to solve.

11) The experiments here mentioned have been carried out on an adequate and synthetic diet with adequate salt content (p. 60), and it has thus been possible to change one or two known factors at a time and thereby to judge of the influence thereof.

12) It is shown that young rats on an A-vitamin-free but otherwise adequate diet develop the well-known symptoms of A-avitaminosis, and it is emphasised that the bone changes produced are *osteoporosis*, even if in some of the animals there are still found preserved only little differentiated cartilage cells in irregular columns in the zone corresponding to the calcification zone.

13) Young rats on an A-vitamin-free and calcium-poor diet (0.04 per cent Ca), as it was to be expected, show hardly any growth. The bones are in a high degree osteoporotic, and this picture is only veiled to a hasty view by the higher formation of still preserved non-differentiated cartilage cells, partly in the epiphyseal line, partly in the marrow cavity.

14) A somewhat similar picture, but with a less violent degree of osteoporosis, may be found in young rats on A-free and phosphorus-poor diet (0.2 per cent P).

15) By numerous experiments on the content of A-vitamin in different butter samples, the butter given as daily additions of 0.025—0.5 gm to our A-free but otherwise adequate diet, it

is found that a quite definite dose is required, differing according to the various sorts of butter, to prevent the occurrence of A-avitaminosis. a) Animals receiving less than such an addition or no addition at all get pronounced *osteoporosis*, b) animals that have received a somewhat greater addition, so that the weight is either kept or rises somewhat, show *pronounced rachitis florida*, but they escape the other symptoms of A-avitaminosis, c) animals receiving such additions that the rise of weight is normal also show *a normal bone picture and normal composition of the bones*.

16) *It is thus proved that rachitis florida can only be produced by supplying certain amounts of A-vitamin.*

The different states of the osseous system: osteoporosis, rickets, normal bone development and growth, are thus consequences of respectively no supply or a subminimal supply of A-vitamin, of some but a suboptimal supply, and finally of an optimal and sufficient supply of A-vitamin. *It is a question of dosage.*

17) Facts absolutely similar to these are shown with respect to cod-liver oil. Here, too, such amounts of cod-liver oil as are sufficient to guarantee a certain growth, but too small to give normal growth, will cause rickets to develop.

18) If osteoporosis has been produced on an A-free diet and the A-free fat is now replaced by adequate A-containing fat, growth of the bone will at once take place and be continued in a normal way.

19) In experiments on the effect of forms of diet containing different amounts of A-vitamin (cod-liver oil) but insufficient salt amounts (partly as regards Ca, partly as regards P) it is found not only by the histological examination, but also by the chemical analyses, that the rachitic picture is pronounced, and that the chemical composition is considerably more abated than if the diet had contained adequate salt mixtures. Further it is shown *that a higher content of A-vitamin in the diet is required to protect the organism against this injurious influence of the deficiency of salt. The increased supply of A-vitamin is only an advantage to the organism, if the salt mixture administered is adequate.*

20) On the basis of the author's own experiments and on the basis of the criticism of the experiments of others it is maintained that there can only be found grounds for the assumption of one fat-soluble, growth-promoting vitamin.

21) This is further shown by experiments with oxidized cod-liver oil. By oxidation the growth-promoting vitamin is destroyed, but at the same time the cod-liver oil has absolutely lost its ability to diminish the water content of the bones and to increase their ash content. The oxidized cod-liver oil showed high »photo-activity« on a photographic plate.

Finally it is also shown that parenteral administration of A-vitamin is able to produce normal bone development.

22) As rickets is thus found only in growing animals, and as rickets must precisely be considered a consequence of the disproportionate growth which occurs when the organism is supplied with certain amounts of A-vitamin which prove insufficient for the normal rate of growth and differentiation, it will be seen *that we can only speak of a real healing of rickets when we are able to increase the processes of differentiation of the growth.* It is here supposed that the other necessary components for the building up of the organism, especially the minerals, are present in the diet in adequate amounts.

23) As the rickety bones develop into osteoporotic bones when the animal has ceased growing or loses weight, it will be understood that a substance or a therapeutic measure can only act »antirachitically«, *healing*, i. e. can only improve the histological formation and chemical composition of the bone, when it is able simultaneously to produce growth processes.

24) It is shown that all symptoms developed in A-avitaminosis: cessation of growth, xerophthalmia, atrophy of the glands, osteoporosis, hypoplasia of the bone marrow, anaemia, the numerous suppurative inflammations occurring in the cavities of the body and the glandular organs, are a simple consequence of the universal cessation of growth. This causes a universal regressive metaplasia of the epithelium in the excretory ducts and in the gland acini, and in this way the entering of

bacteria is simplified and inflammations often commence. All these symptoms vanish promptly upon supplying A-vitamin.

25) Finally the author discusses the importance of the results obtained for the therapy and prophylaxis of the A-avitaminosis, and especially for rickets in children.

VII.

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VIII.
MICROPHOTOGRAPHS.



Fig. 1. R 39, kept on N-diet with 15 per cent butter fat. Weight rose from 47—100 gm. Age, 80 days. Normal costa. A normal zone of proliferous cartilage cells is seen, which towards the marrow cavity is completed by a distinct and even zone of cartilage cells. These show the type of »great vesicular hyperplasia«. From here proceed immediately distinctly calcified ossification direction lines. Spongy bone trabeculae are found. Marrow cells present in great amounts. Magn. $\times 40$.



Fig. 2. C 24, kept on $\div$ A-diet. Weight rose from 35—88 gm. Age, 78 days. Osteoporotic costa. The proliferous zone is seen to be considerably narrow, no »great vesicular hyperplasia«. Complete cessation of growth. No ossification direction lines. Scanty but well calcified spongy bone trabeculae.

No osteoid tissue. Magn. $\times$ 40.

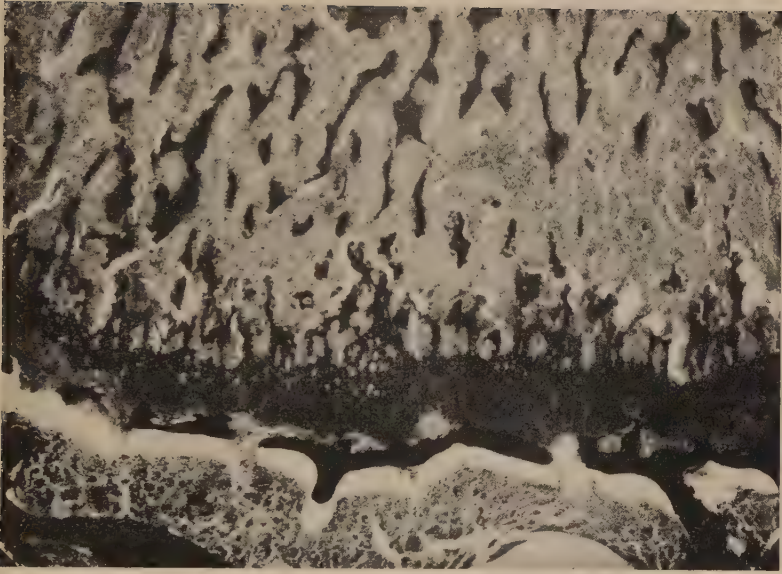


Fig. 3. C 24, Osteoporotic tibia. Proliferous zone narrow, some non-differentiated cartilage cells towards the marrow cavity. The matrix is calcified. The scanty spongy trabeculae are calcified and without osteoid borders. The calcified matrix is silver impregnated. Magn. $\times 40$.

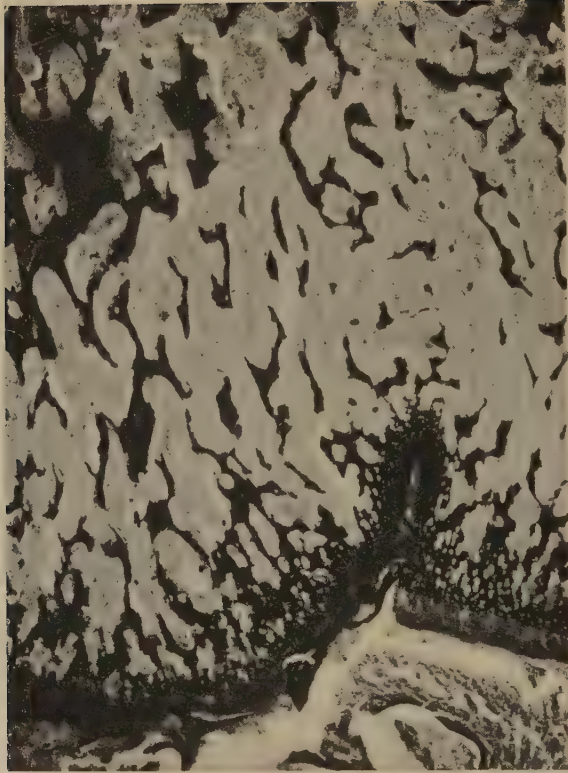


Fig. 4. P 45, kept on $\div$ A-diet plus 1 mgm cod-liver oil. Weight rose from 25—37 gm. Age, 68 days. Osteoporotic femur epiphysis. Arrested growth is seen in the proliferation zone, scanty spongy trabeculae. Enlarged blood-vessels in the marrow cavity. No osteoid tissue. Silver impregnation.

Magn. $\times$ 40.



Fig. 5. P 48, kept on $\div$ A-diet plus 1 mgm cod-liver oil. Weight rose from 25—44 gm. Age, 78 days. Considerable osteoporosis in costa. Marked cessation of growth in the proliferous zone. No ossification direction lines. No spongeous trabeculae. Silver impregnation. Magn. $\times$ 40.



Fig. 6. P 39, kept on $\div$ A-diet plus 20 mgm oxidated cod-liver oil. Weight rose from 36—86 gm. Age, 116 days. P 39 belongs to another litter than P 45 and P 48. Considerable osteoporosis in costa. Complete cessation of growth in the proliferation zone. No ossification direction lines, only few spongy trabeculae. No osteoid tissue. Few marrow cells and slight polymorphia. Silver impr. Magn. $\times 40$.

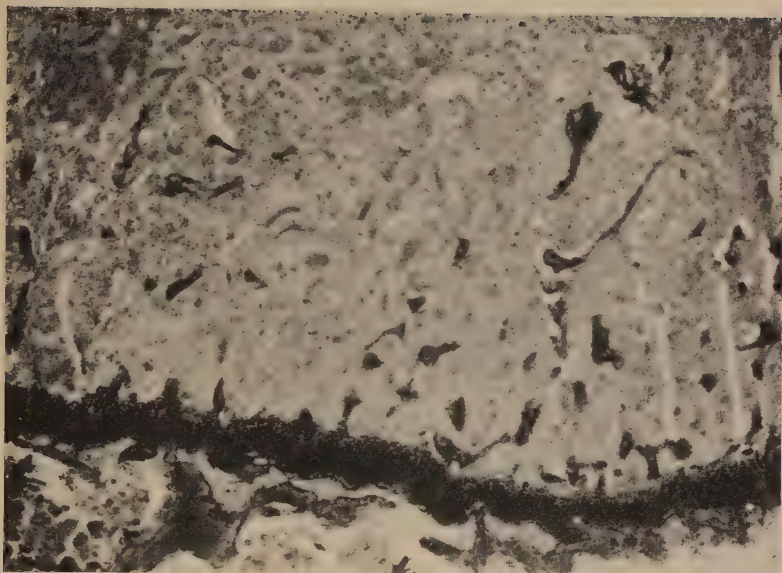


Fig. 7. R 62, kept on $\div$ A-diet and after commencement of cessation of growth addition of 0.1 gm butter. This was insufficient to promote growth. Weight rose from 42—53 gm. Age, 81 days. Considerable osteoporosis in femur. Complete cessation of growth in the epiphyseal line. No ossification direction trabeculae. Only a few remains of spongy trabeculae. Hypoplasia of marrow tissue. Enlarged blood-vessels. Haem.-eosin. Magn. $\times 40$.



Fig. 8. D 29, kept on $\div$ A-diet plus 0.025 gm butter D II. Weight rose from 37—76 gm. Age, 101 days. Osteoporotic costa. But it will be observed that the cessation of growth in the cartilage proliferous zone is not complete, as shorter and longer columns of more or less differentiated cartilage cells are found some with intact cartilage capsule, others with open capsule. Some lie in calcified, others in uncalcified cartilage matrix. No spongy trabeculae are found. The marrow cells are pronouncedly monotypic. Silver-impr. Magn. $\times 40$.



Fig. 9. D 10, kept on $\div$ A-diet plus 0.05 gm butter D I. Weight rose from 44—109 gm. Age 158 days. Costa with considerable rickets. Considerable enlargement of the proliferation zone with non-differentiated not demolished cartilage cells, which reach high up into the marrow cavity. The cartilage cells lie partly in a calcified and partly in an uncalcified matrix. Spongy trabeculae are found in rather plentiful amounts, but around the calcified silver stained middle of the trabeculae thick borders of uncalcified, osteoid tissue. Silver-impr. Magn. $\times 40$.

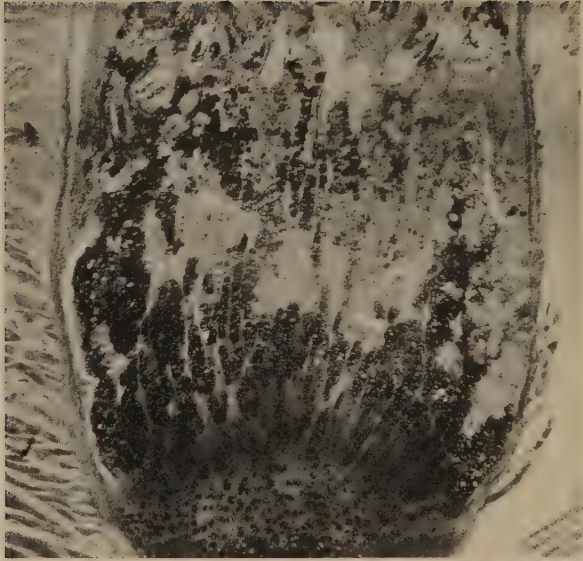


Fig. 10. D 11, kept on $\div$ A-diet plus 0.05 gm butter D II. Weight rose from 45—118 gm. Age, 158 days. Costa with considerable rickets. Considerable enlargement of cartilage proliferation zone with preserved little differentiated cartilage cells high up in the marrow cavity. The cartilage matrix is calcified in several places, in other places uncalcified. Silver-impr. Magn. $\times$ 40.

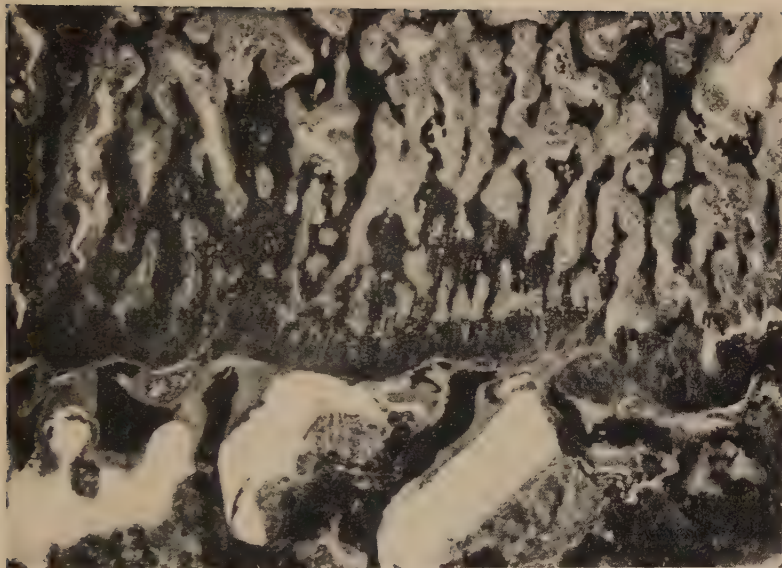


Fig. 11. D 11, femur epiphysis with distinct rickets. Irregular epiphyseal line, preserved cartilage cells in the marrow cavity. Thick grayish borders of osteoid tissue round about the calcified silver stained middle of bone trabeculae in the marrow cavity as well as downwards in the epiphysis.
Silver-impr. Magn. $\times 40$.

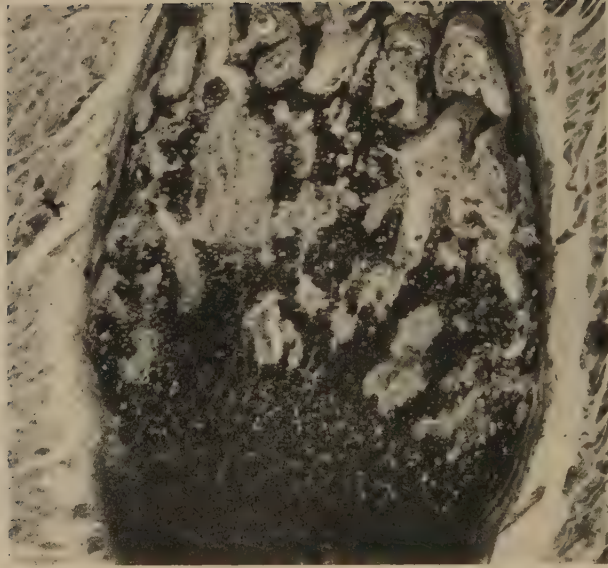


Fig. 12. D 14, kept on $\div$ A-diet plus 0.05 gm butter D II. Picture similar to D 11, but the osteoid borders somewhat more obvious. Silver impr. Magn. $\times$ 40.



Fig. 13. D 4, kept on $\div$ A-diet plus 0.05 gm butter D III. Picture similar to fig. 10. Silver impr. Magn. $\times 40$.



Fig. 14. D 3, kept on ÷A-diet plus 0.1 gm of butter D I. Weight rose from 34—108 gm. Age, 162 days. Costa with distinct rickets. The cartilage proliferation zone is enlarged and irregular. Preserved non-differentiated cartilage cell columns and isles are found in the marrow cavity. The cartilage cells lie in some places in uncalcified matrix, in other places in distinctly calcified matrix. Grayish osteoid borders around the silver-stained spongy trabeculae. The blood vessels enlarged. Silver-impr.

Magn. $\times 40$.



Fig. 15. D 3, Costa with symptoms of rickets, but the changes in this rib are less pronounced than in that mentioned above from same animal.
Silver impr. Magn. $\times 40$.

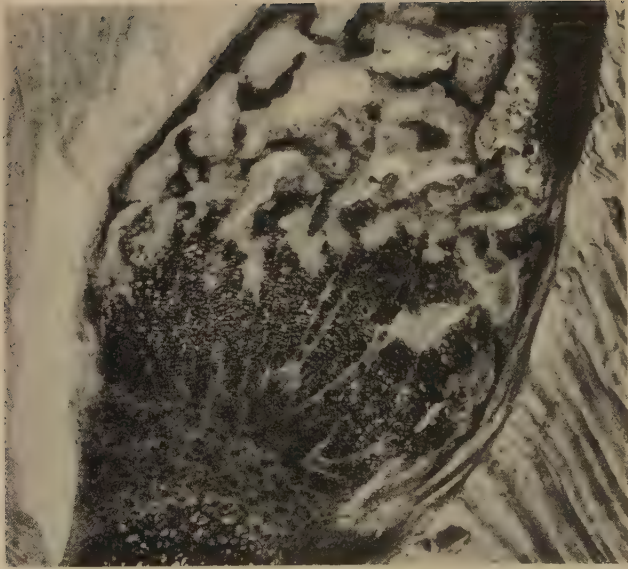


Fig. 16. D 5, kept on $\div$ A-diet plus 0.1 gm butter D II, weight rose from 35—115 gm. Age, 132 days. Distinct rickets in costa. Rosary is found. Considerably enlarged proliferation zone. The cartilage matrix mainly calcified, but in a few places uncalcified. Osteoid borders on the spongy trabeculae. The blood vessels in the marrow cavity considerably enlarged. Silver-impr. Magn. $\times 40$.

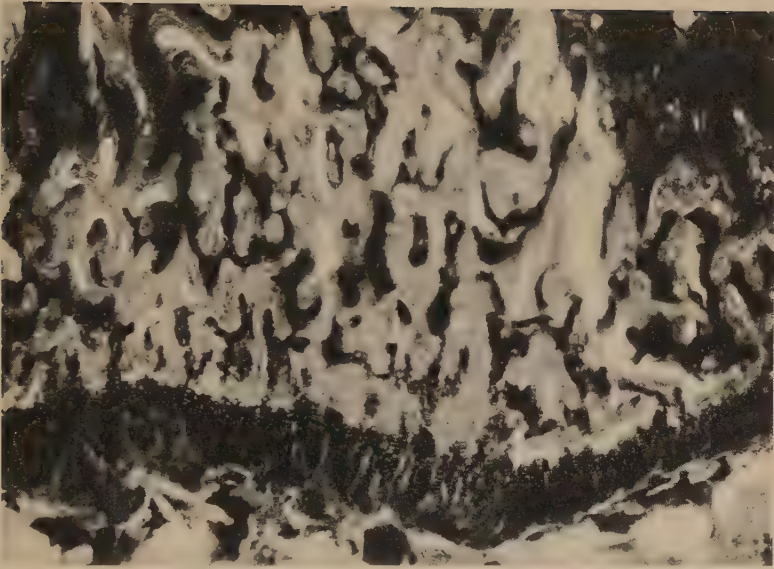


Fig 17. D 5, Femur epiphysis. An irregular epiphyseal line is seen. Preserved non-differentiated cartilage cells in the marrow cavity. No distinct ossification direction lines. Some osteoid tissue on the spongy trabeculae. Enlarged blood vessels in the marrow cavity. Silver impr. Magn. $\times 40$.

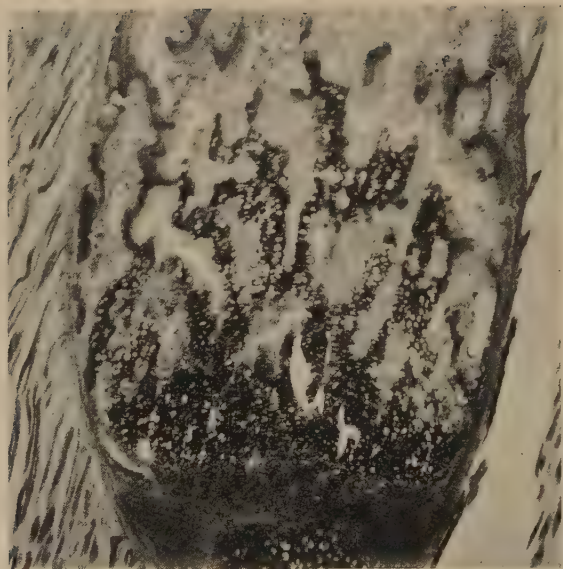


Fig. 18. D 6, kept on $\div$ A-diet plus 0.1 gm butter of D I. Weight rose from 35—115 gm. Age, 168 days. Costa with pronounced rickets. Preserved and little differentiated cartilage cells high up in the marrow cavity. Considerable irregularity of the provisional calcification zone. The cartilage cells are found surrounded by a calcified matrix, but in some places the matrix is uncalcified. Osteoid tissue around the spongy trabeculae.

Silver impr. Magn. $\times$ 40.

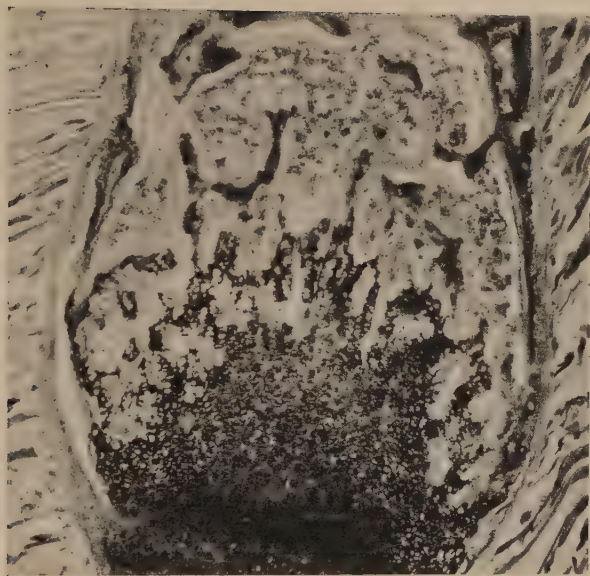


Fig. 19. D 7, kept on $\div$ A-diet plus 0.1 gm butter D III. Costa with pronounced rickets. Same description as at Fig. 18 and 16. Silver impr.
Magn. $\times$ 40.

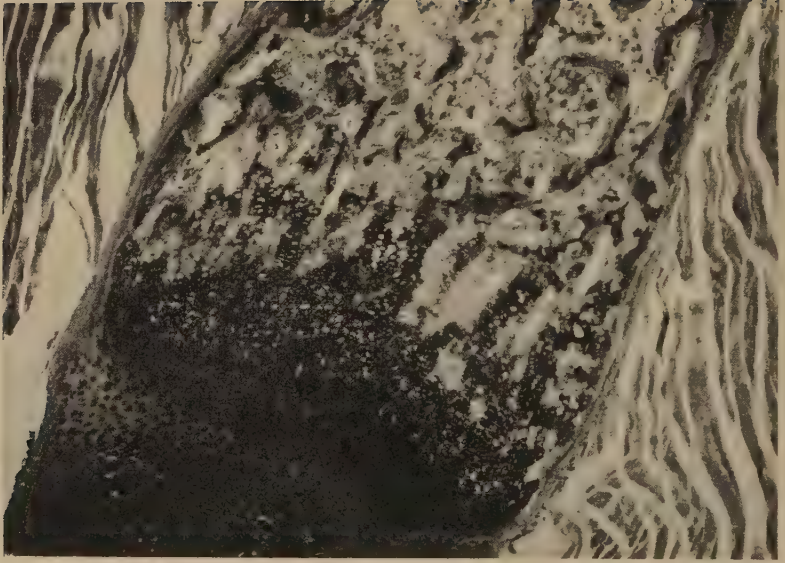


Fig. 20. D 16, kept on $\div$ A-diet plus 0.15 gm butter D II. Pronounced rickets in costa. Description exactly the same as in fig. 18 and 16. Weight rose from 35—109 gm. Age, 157 days. Silver impr. Magn. $\times$ 40.



Fig. 21. R 66, kept on $\div$ A- $\div$ P-diet. Weight rose from 31—48 gm. Age, 68 days. Femur epiphysis, the lower part of the marrow cavity of femur filled up with a considerable accumulation of non-differentiated cartilage cells. These lie in a partly calcified, partly uncalcified matrix. The spongy trabeculae are few and thin. Silver impr. Magn. $\times 40$.



Fig. 22. R 86, kept on $\div$ A-diet with 15 per cent of olive oil and 0.02 per cent of cod-liver oil. Weight rose from 55—82 gm. Age, 83 days. Rickets in costa. An enlarged proliferation zone is seen and enlarged provisional calcification zone, in which is found some non-differentiated cartilage cell columns and isles. Narrow osteoid borders around the scanty spongy trabeculae. Silver impr. Magn. $\times 40$.



Fig. 23. R 111, kept on $\div$ A- $\div$ Ca-diet with 15 per cent olive oil. Weight rose from 48—72 gm. Age, 70 days. Rickets in costa. A very considerable broad proliferation zone with clear non-differentiated cartilage cells is seen. These show an uneven limit by the marrow cavity and in this are found scattered isles of cartilage cells in a partially calcified matrix. Broad borders of osteoid tissue in the marrow cavity. Silver impr. Magn.

× 40.

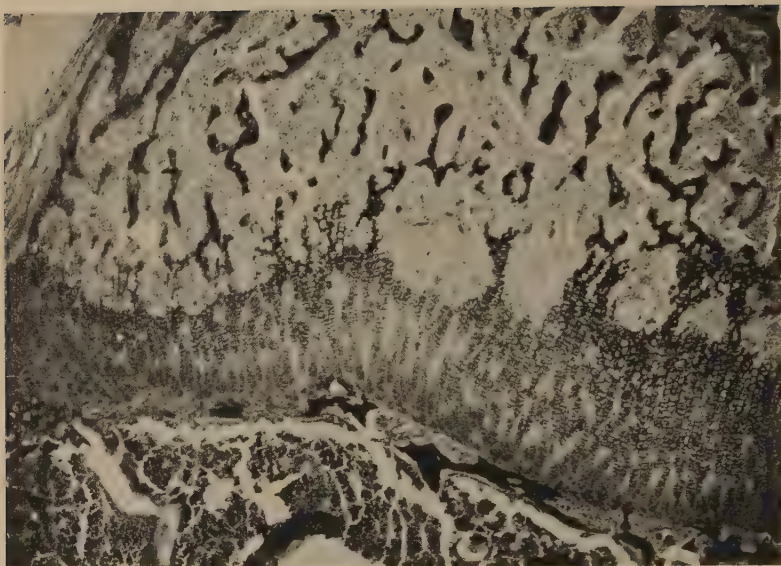


Fig. 24. R 113, kept on $\div$ A- $\div$ Ca-diet + 0,02 p.c. of cod-liver oil. Weight rose from 46—67 gm. Age, 70 days. Femur epiphysis with distinct, but osteoporotic rickets. The cartilage cells in the epiphyseal line have remained as a broad heap. The cells have a very non-differentiated character, and a very uneven limit towards the marrow cavity. The spongy trabeculae are few and thin and all of them are surrounded by osteoid tissue. Silver impr. Magn. $\times$ 40.

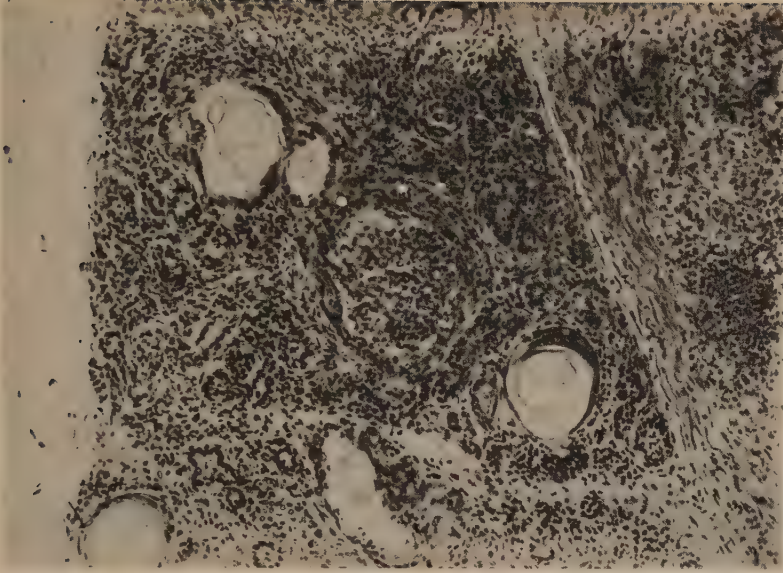


Fig. 25. D 31. Preparation of the submaxillary gland. A rather diffuse inflammation of this part of the gland is seen, with a considerable accumulation of lymphocytes. In the middle of the picture is found an excretory duct filled up with purulent secretion and desquamated cells. Around this are seen several ducts in which the epithelia have undergone a marked metaplasia. Instead of the single layer of cylinder cells which normally cover the luminary, plate-epithel-like cells are found here in several layers (like stratified epithelium), the inner ones are dead, the nucleus has diminished and the luminary is filled up with a keratin-like mass. Magn.

× 190.

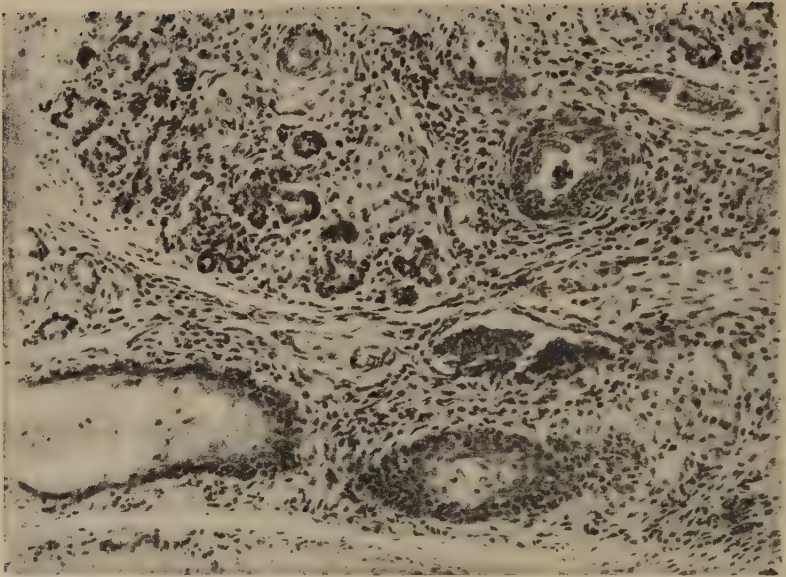


Fig. 26. P 59. A part of the submaxillary gland is seen. Single lobular ducts and serous tubuli are still recognizable. The gland is otherwise very atrophic and there are some scanty leuco- and lymphocyte-infiltrations. Several lobar ducts and excretory ducts with marked metaplasia of the epithelia are seen, as well as in some places blocking of the luminary with keratinized stratified epithelium cells. Magn. $\times$ 190.

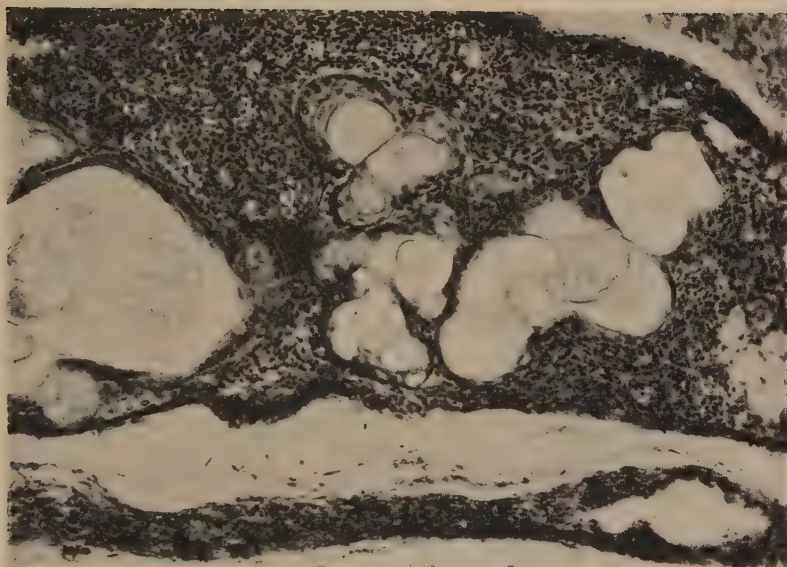


Fig. 27. D. 31. Shows similar phenomena to those described above.
Magn. $\times 190$.



*Fig. 28. D 31. Shows similar phenomena to those mentioned above. Magn.
× 190.*

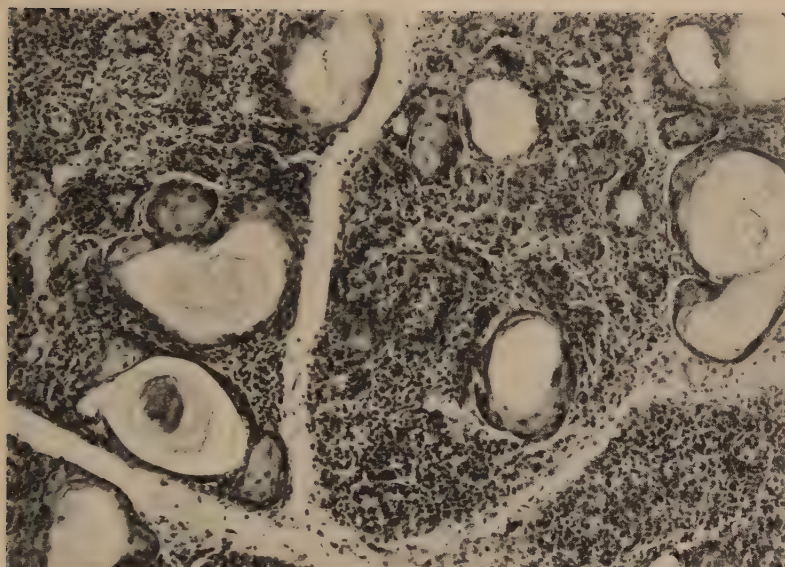


Fig. 29. D 31. The submaxillary gland, several lobuli of the gland with dilated lobar ducts and lobular ducts and some serous tubuli. The cylindric epithelium in the lobar ducts and in the lobular ducts has undergone a considerable metaplasia and the heaped up flat epithelium cells in certain places fill up the lumen. Furthermore, in the lumen of the greater ducts is found keratin-like blocking. Also in this part of the gland is found inflammation. Magn. $\times 190$.



Fig. 30. P 51. Excretory duct from the submaxillary gland. In the lower left corner is seen the mucous part of the gland, at top to the left is seen a ganglion with big ganglic cells. No obvious inflammation in the gland. In the middle of the picture is seen a great excretory duct with metaplasia of the epithelium. Blocking of the epithelium cells and some desquamation. No inflammation. Magn. $\times 190$.

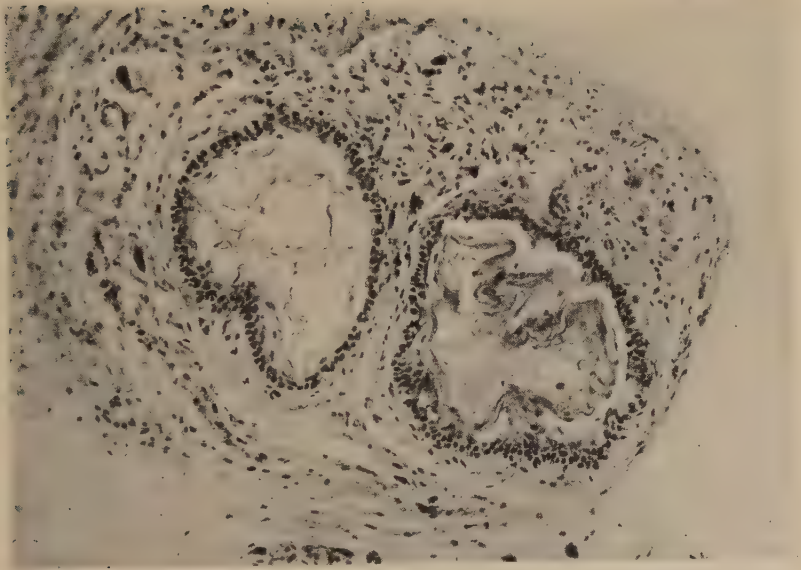


Fig. 31. P 45. Two great excretory ducts from the parotis gland are seen. The cylinder epithelium is flattened, has undergone metaplasia. The epithelium cells are heaped upon each other, there is some desquamation and lumina are filled up with a keratin-like mass. There is hardly any inflammation. On the extreme right is found a ganglion. Magn. $\times 190$.

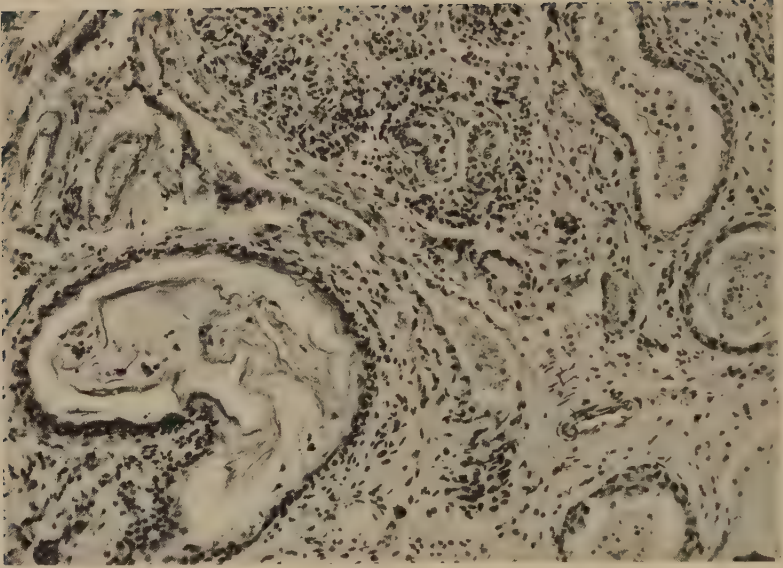


Fig. 32. P 45. Part of the submaxillary gland with a single recognizable part of the serous part of the gland. Several great excretory ducts with distinct metaplasia of the epithelium are seen. Hardly any inflammation.
Magn. $\times 190$.

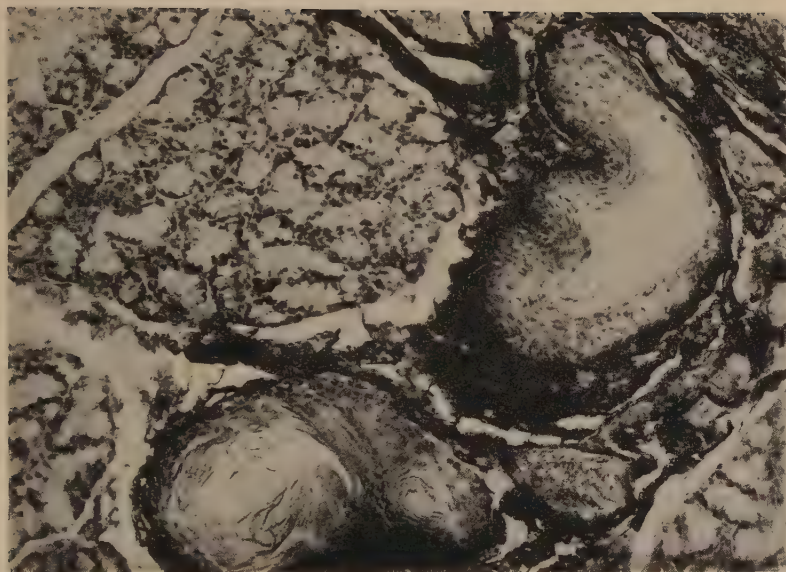


Fig. 33. D 56. Part of the submaxillary. Three lobuli are seen of the mucous part of the gland enclosing two great excretory ducts. In these is found a considerable accumulation of »stratified« epithelium cells, which have undergone metaplasia. They fill up great parts of lumen, the outermost are keratinized, show blurred cell limits, waste of the nucleus and transformation of the protoplasm to a refractive keratin-like substance, which partly fills up the lumen. No inflammation. Magn. $\times 190$.

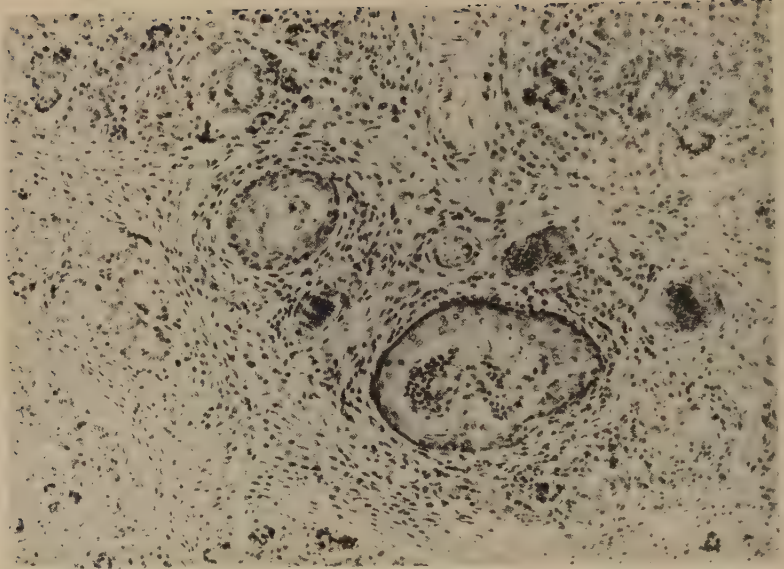


Fig. 34. P 59. Part of the submaxillary gland. Two lobar ducts, the epithelium of which has undergone metaplasia. The epithelial cells are very flattened, lie heaped upon each other and show no marked cell limits, the inner cells have less stained nucleus than the more periferous on the basal membrane. Lumen in the upper duct is partly filled with desquamated flat epithelial cells. Lumen in the lower duct is partly filled with a secretion consisting of lymphocytes and leucocytes. Magn. $\times 190$.

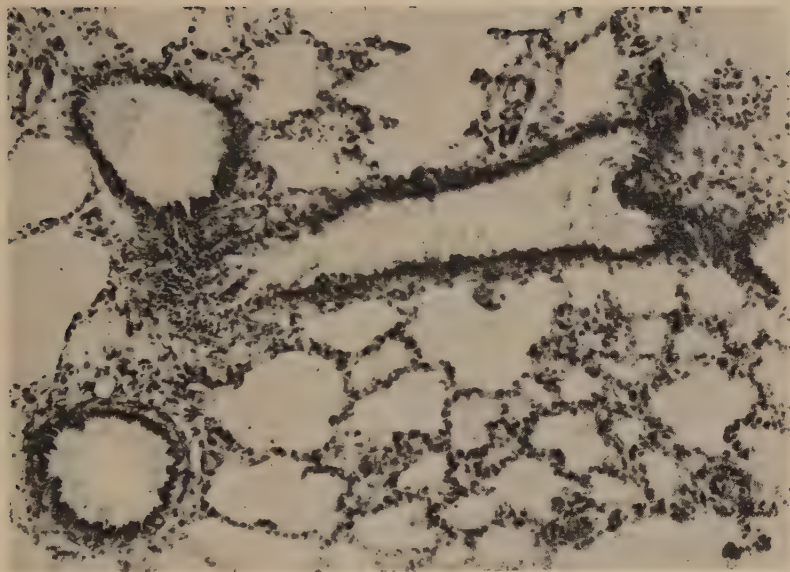


Fig. 35. P 41. Bronchial lumina without symptoms of inflammation, but with metaplasia of the epithelium and some desquamation. Magn. $\times$ 190.

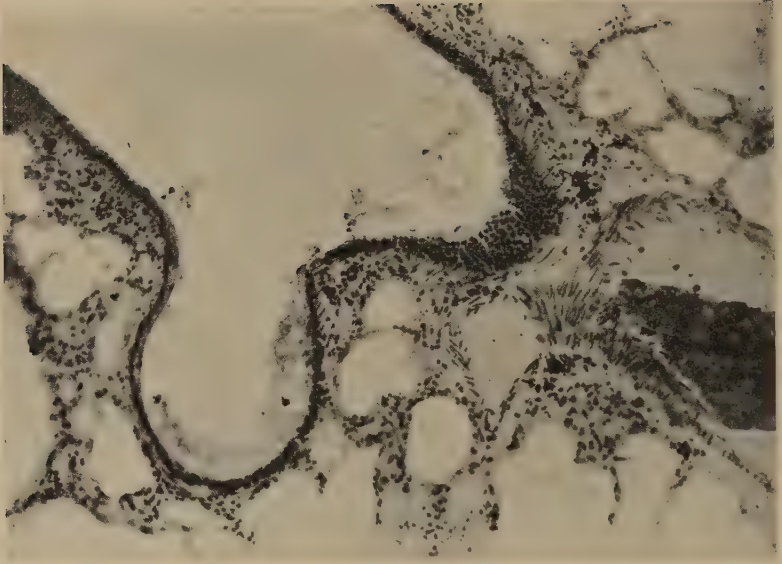


Fig. 36. D 31. A bronchial lumen with partly flattened, atrophic epithelial cells, partly change of the epithelial cells by metaplasia. Hardly any inflammation. Magn. $\times 170$.

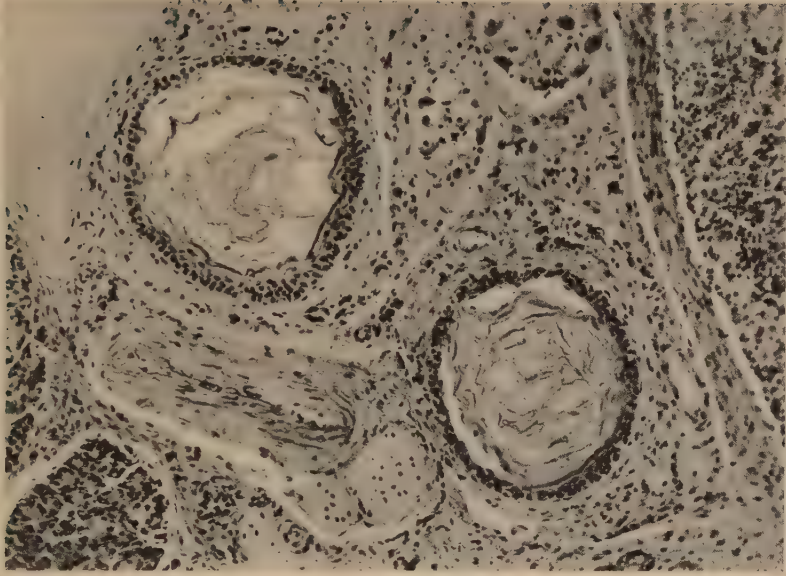


Fig. 37. P 45. The picture shows great excretory ducts with metaplasia of the epithelial cells and obstruction of the lumen by keratinized masses. To the left two serous lobuli of the parotid gland. On the extreme right, part of a lymphatic gland. In the middle furthermore an artery, two nerves and two ganglions. Magn. $\times 170$.

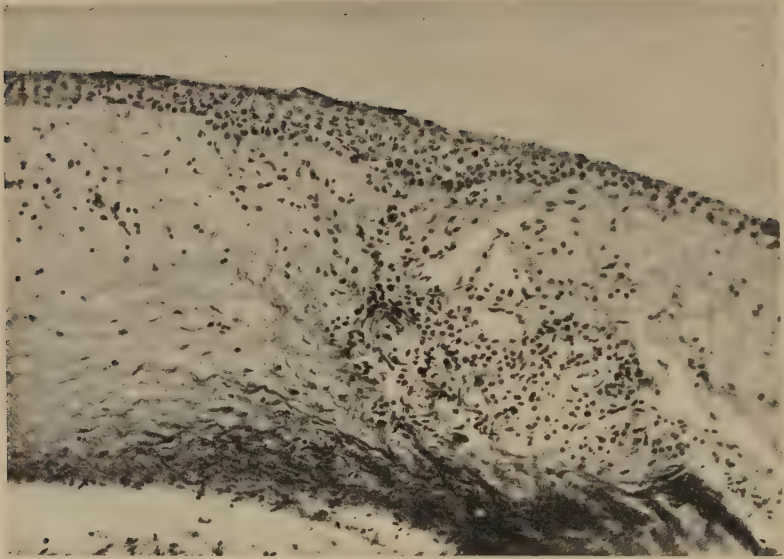


Fig. 38. P 41. Cornea, to the right is seen stratified epithelium of cornea. To the right and to the left the epithelium layer is low and the cells are flattened. In the middle is some thickening of the epithelium cells and unevenness of the surface, the cells are here keratinized, and some desquamation is found. Downwards is some immigration of leucocytes into substantia propria and some enlargement of the cell spaces. Magn. $\times 170$.

IX.
WEIGHT-CURVES.

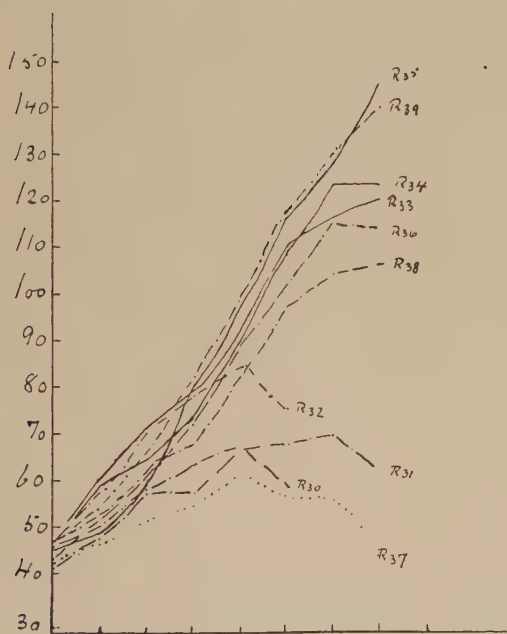


Fig. I.

- ÷ A-diet (lard) R 37.
- . - . - . ÷ A-diet (olive oil) R 30, R 31, R 32.
- . - - - - + A-diet (15 p. c. butter fat) R 36, R 38, R 39.
- + A-diet (an unsaponifiable residuum of cod-liver oil dissolved in olive oil) R 33, R 34, R 35.

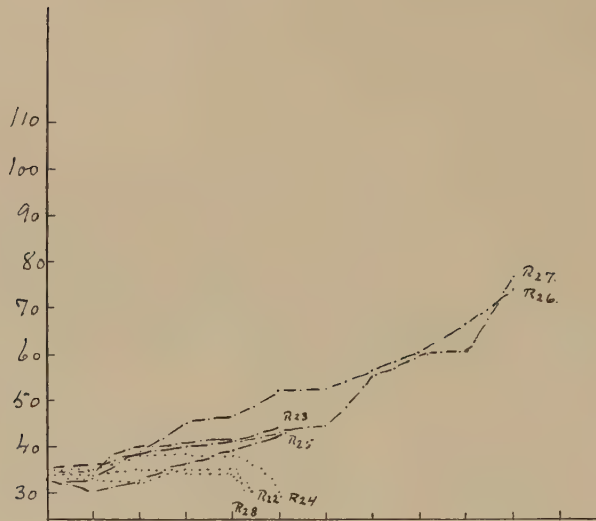


Fig. II.

..... $\div$ A $\div$ Ca-diet R 22, R 24, R 28.

— . — . — . $\div$ A $\div$ Ca-diet + cod-liver oil R 23, R 25, R 26, R 27.

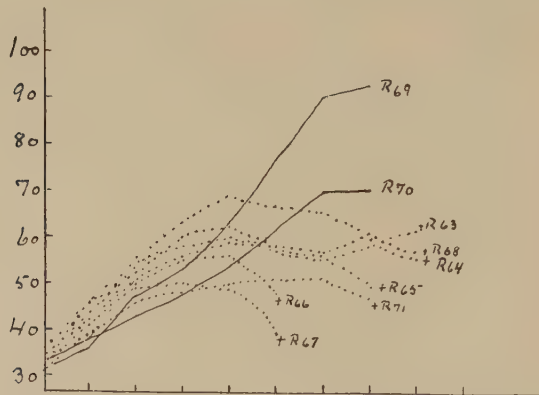


Fig. III.

$\div$ A $\div$ P-diet, R 69, R 70 + 3 p. c. of cod-liver oil.

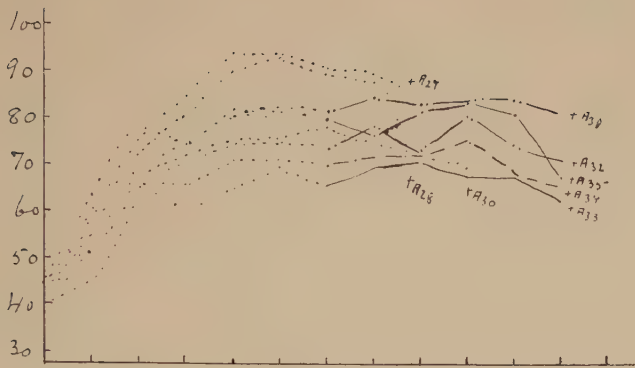


Fig. IVa.

..... lard
 — I butter group A 0.05 gm daily.
 .. II
 . — . III

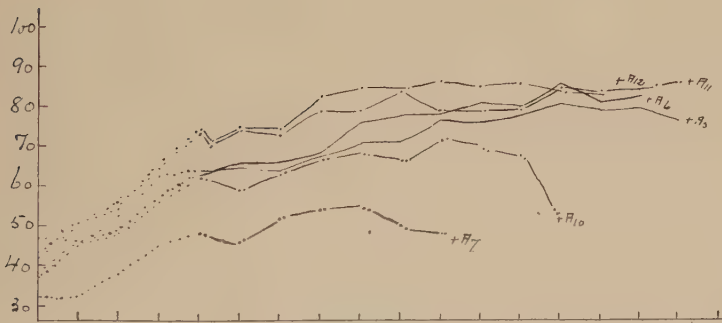


Fig. IVb.

..... lard
 — I butter group A 0.10 gm daily.
 .. II
 . — . III

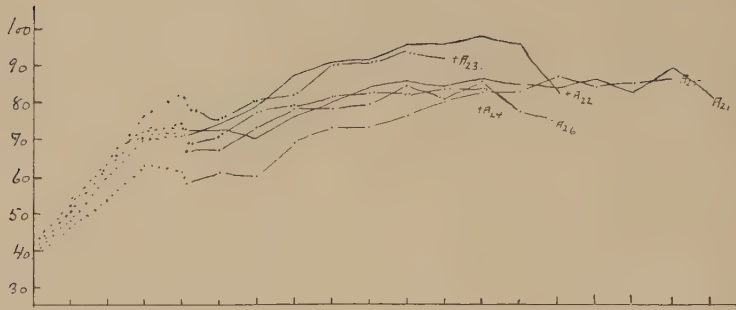


Fig. IVc.

..... lard
 — I butter group A 0.15 gm daily.
 - - - II
 - - - III

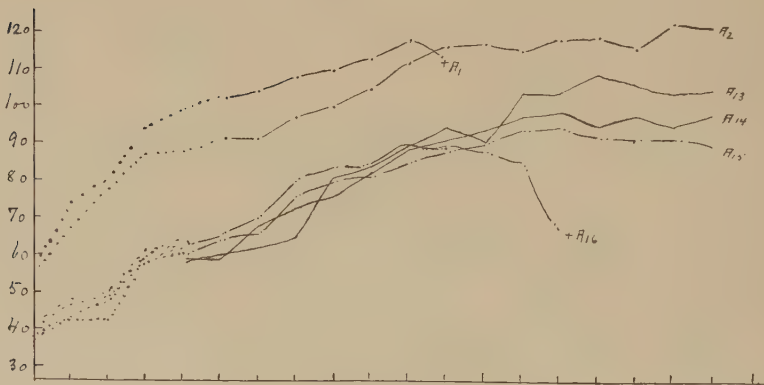


Fig. IVd.

..... lard
 — I butter group A 0.20 gm daily.
 - - - II
 - - - III



Fig. IVe.

..... lard
 — I butter group A 0.30 gm daily.
 - - - II
 . - . - . III

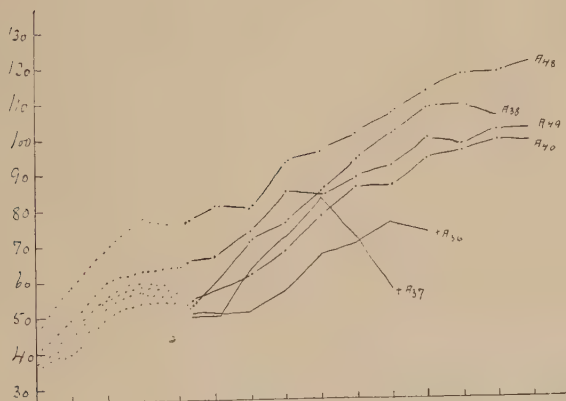


Fig. IVf.

..... lard
 — I butter group A 0.40 gm daily.
 - - - II
 . - . - . III

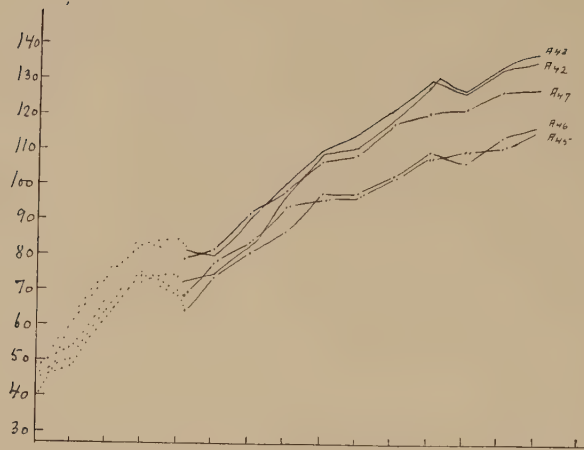


Fig. IVg.

..... lard
 ————— I
 .. ———.. II
 . — — . — . III
 butter group A 0.50 gm daily.

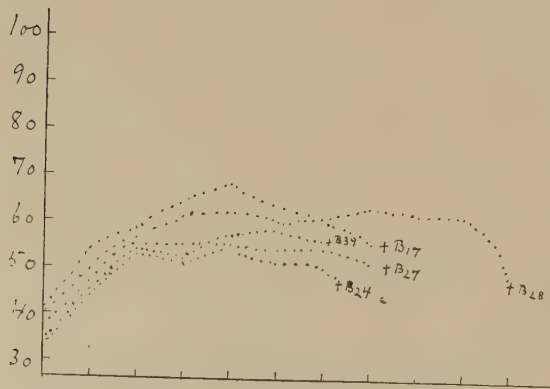


Fig. V.

..... lard (no addition)

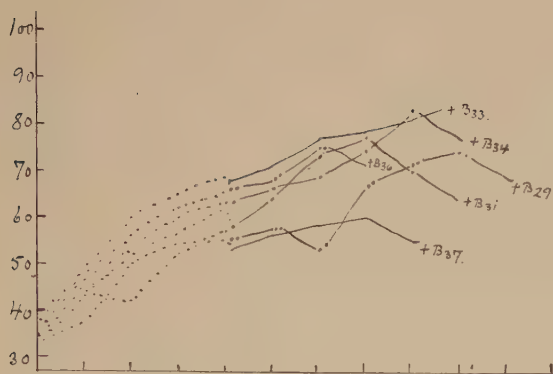


Fig. Va.

..... lard
 — I butter group B 0.025 gm daily.
 .. II
 . — . III

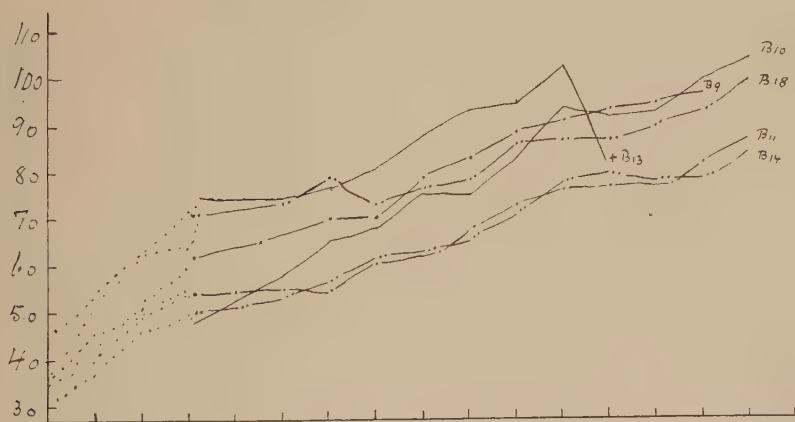


Fig. Vb.

..... lard
 — I butter group B 0.05 gm daily.
 .. II
 . — . III

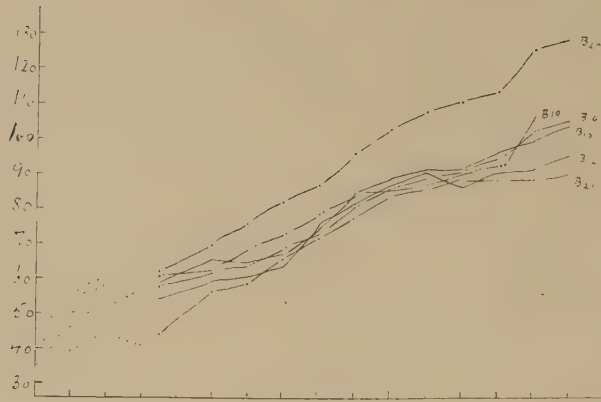


Fig. Vc.

..... lard
 — I butter group B 0.1 gm daily.
 .. II
 III

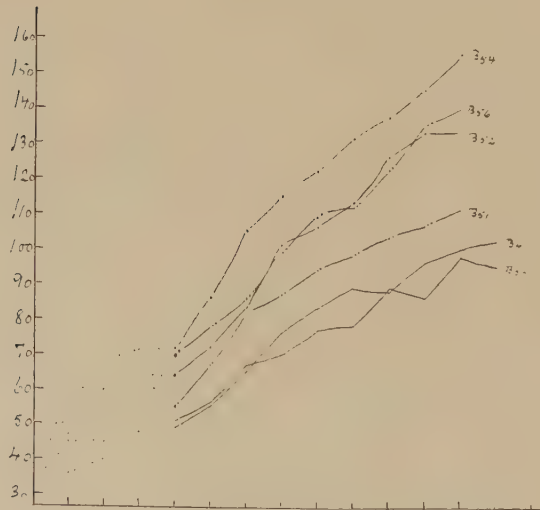


Fig. Vd.

..... lard
 — I butter group B 0.15 gm daily.
 .. II
 III

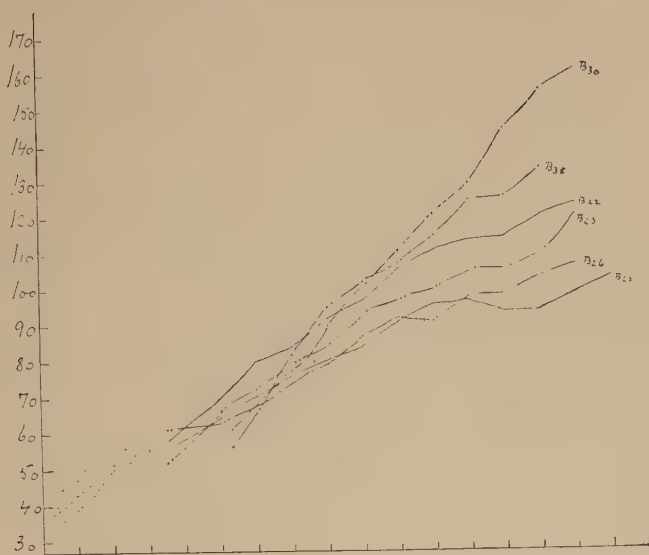


Fig. Ve.

..... lard
 ————— I butter group B 0.2 gm daily.
 - . - . - . II
 - - - - - III

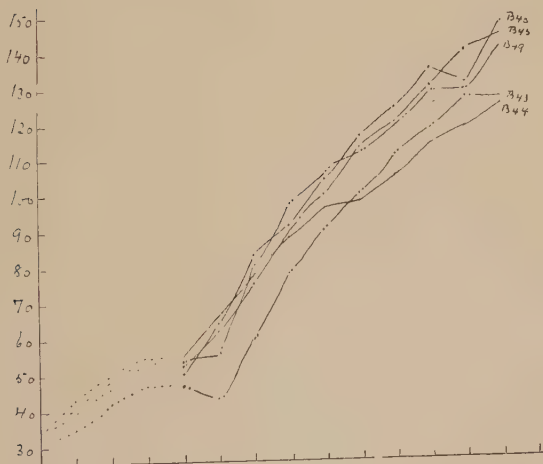


Fig. Vf.

..... lard
 ————— I butter group B 0.3 gm daily.
 - . - . - . II
 - - - - - III

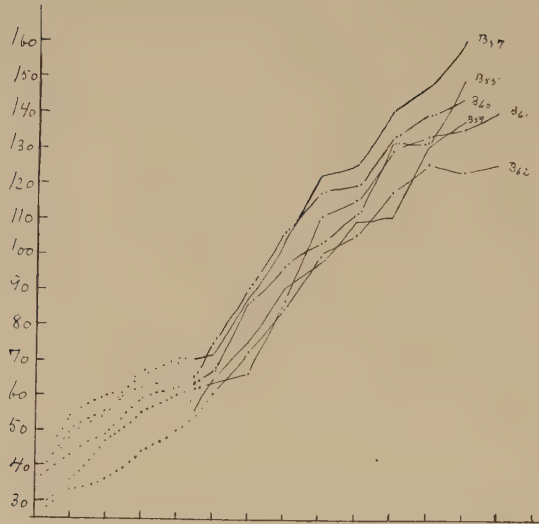


Fig. Vg.

..... lard
 — I butter group B 0.4 gm daily.
 .. II
 . — . III

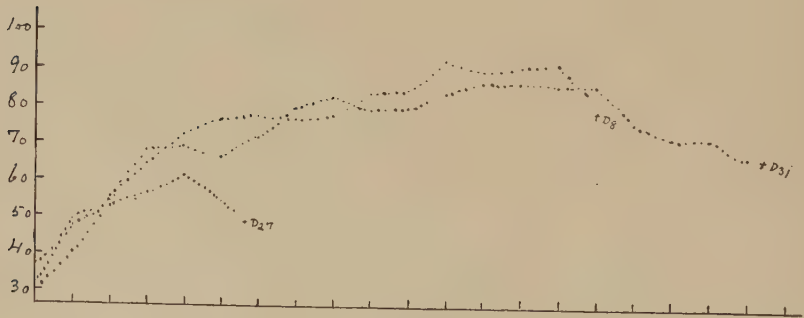
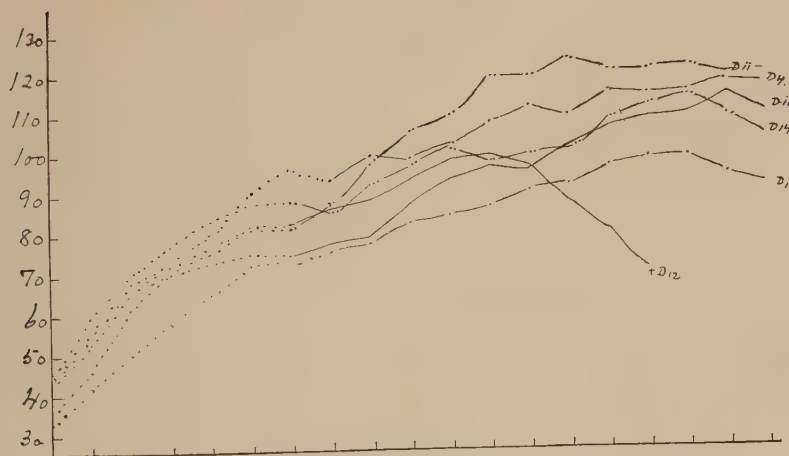
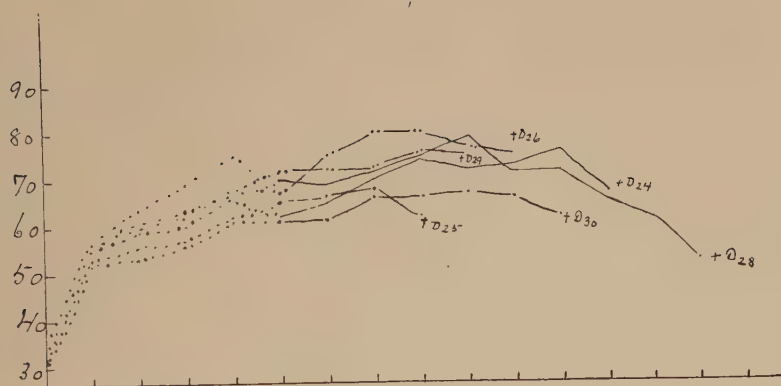
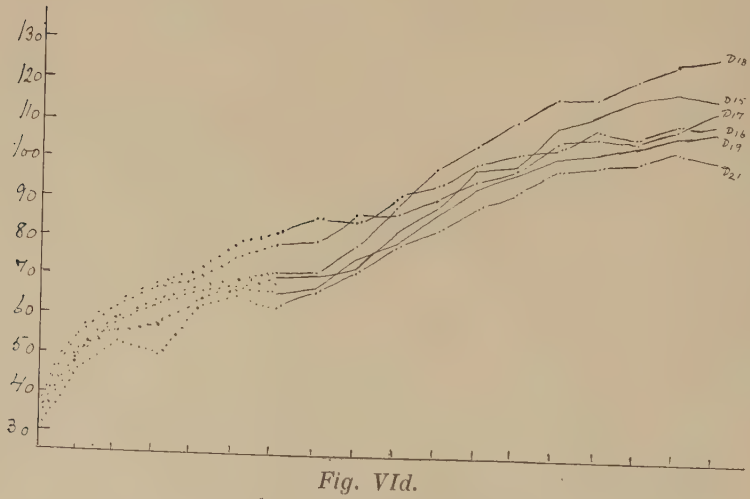
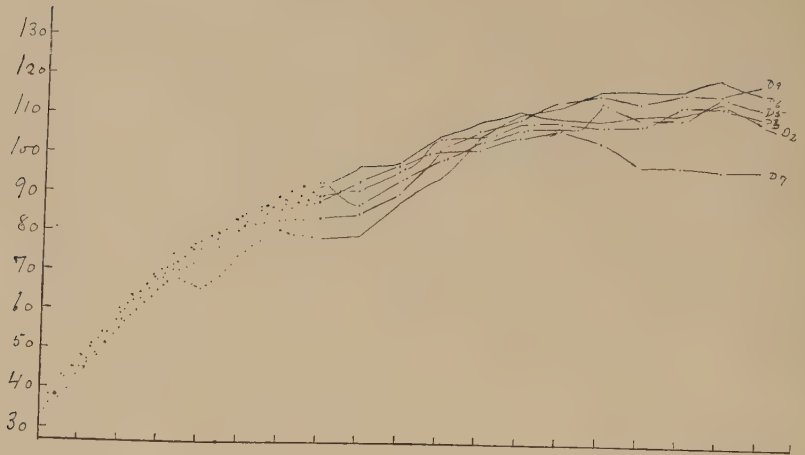


Fig. VI.

..... lard (no addition)





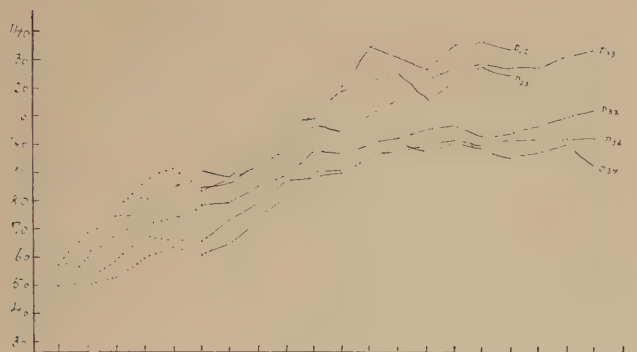


Fig. VIe.

..... lard
 ————— I butter group D 0.2 gm daily.
 II
 . — . — . III

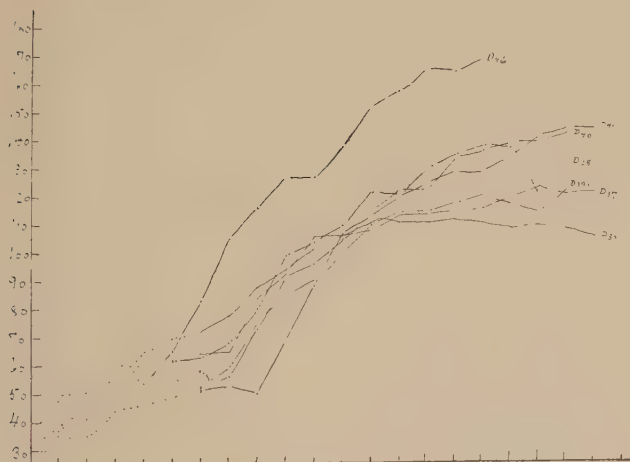


Fig. VI f.

..... lard
 ————— I butter group D 0.3 gm daily.
 II
 . — . — . III

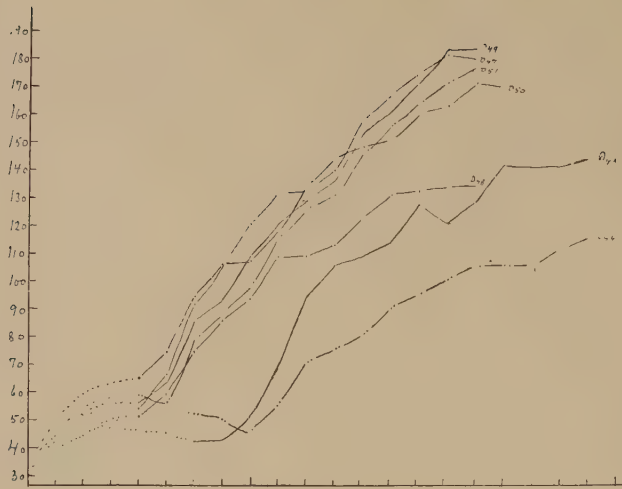


Fig. VIg.

..... lard
 — I butter group D 0.4 gm daily.
 - · - · - II
 - - - - - III

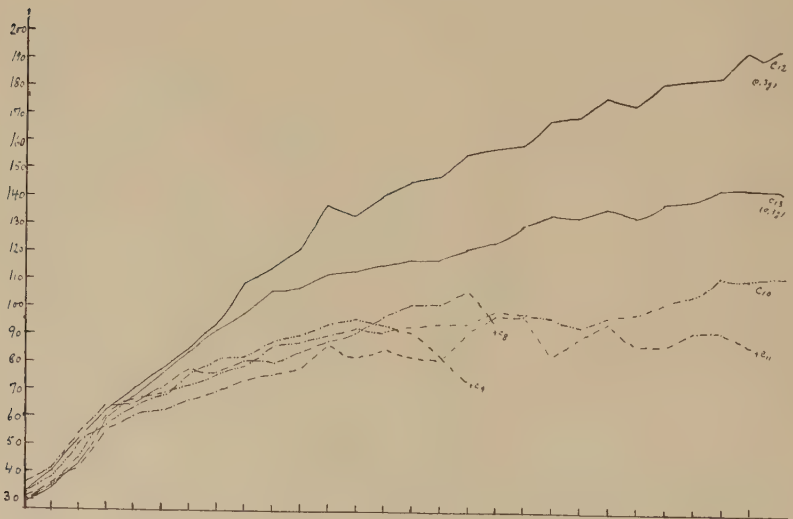


Fig. VIIa.

..... lard
 - · - · - 0.025 gm of butter group B I from the beginning of the experim.
 - · - · - 0.05 "
 — 0.3 "
 - - - - - 0.4 "

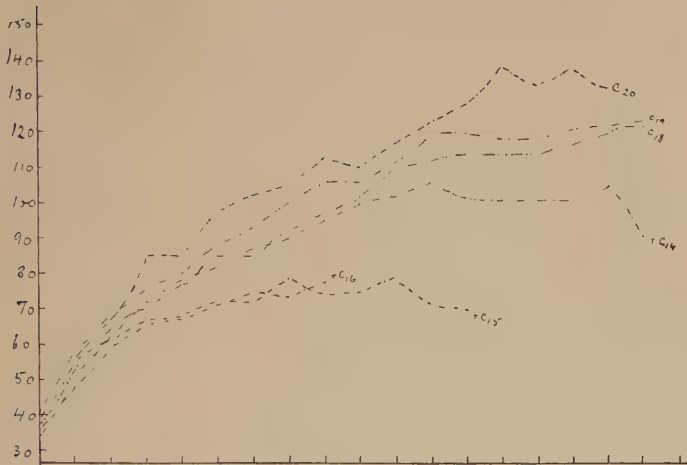


Fig. VIIb.

- lard
 0.025 gm of butter group B I from the beginning of the experim.
 0.05 "
 0.1 "
 0.2 "

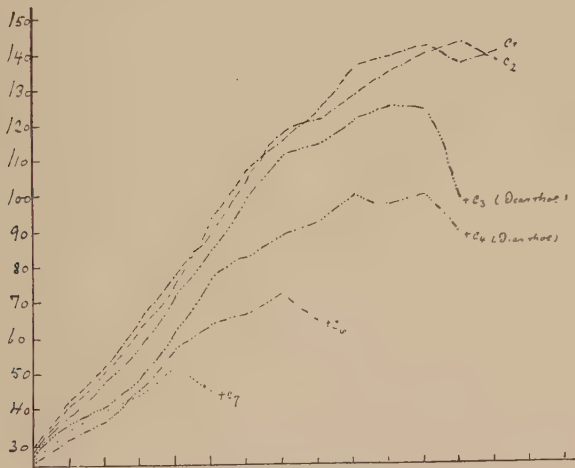


Fig. VIIIa.

- 0.025 gm of butter group D I from the beginning of the experim.
 0.05 "
 0.1 "
 0.2 "

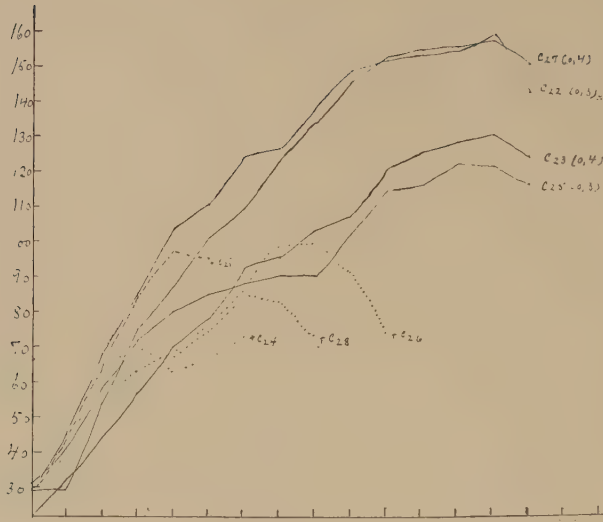


Fig. VIIIb.

- lard
 - . - . . 0.2 gm of butter group D I from the beginning of the experim.
 ——— 0.3 or 0.4 gm daily.

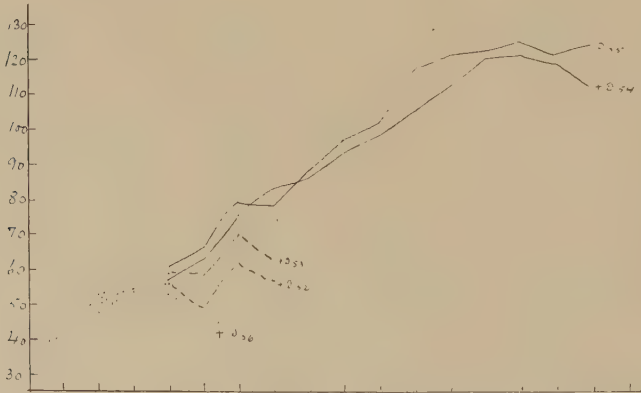


Fig. IX.

- lard
 - . - . . 0.1 gm control-experiment with butter group A stored for one year in a refrigerator.
 ——— 0.2 "

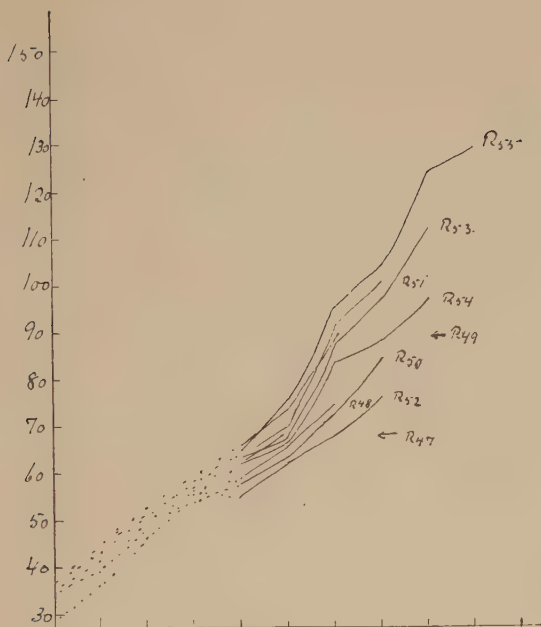


Fig. X.

..... ÷ A-diet (—— later 15 p. c. butter fat).

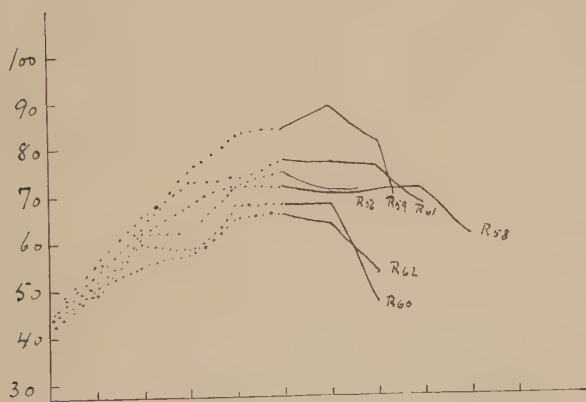


Fig. XIa.

o. l. gm butter after cessation of the gain in weight.

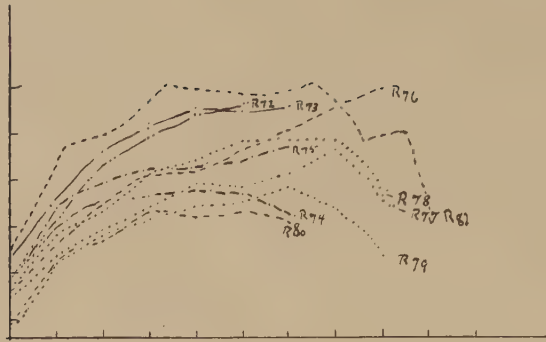


Fig. XIb.

÷ A-diet + 0.10 gm butter daily. R 80, R 81 ÷ A-diet without addition of butter.

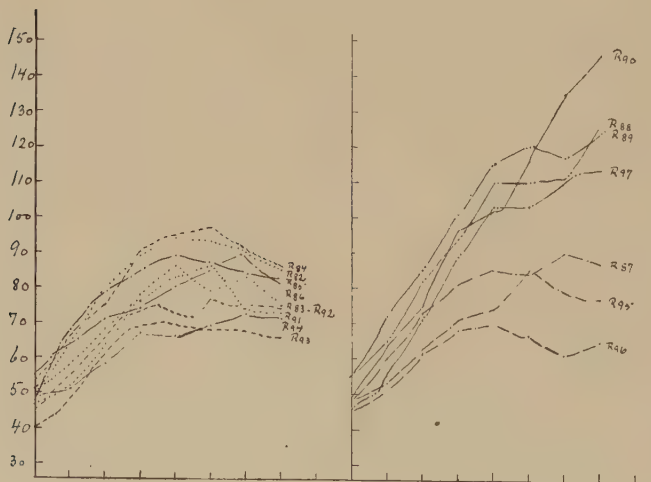


Fig. XIIa.

÷ A-diet, olive oil with varying additions of cod-liver oil. groups I—III.

Fig. XIIb.

÷ A-diet, olive oil with varying additions of cod-liver oil. groups IV—VI.

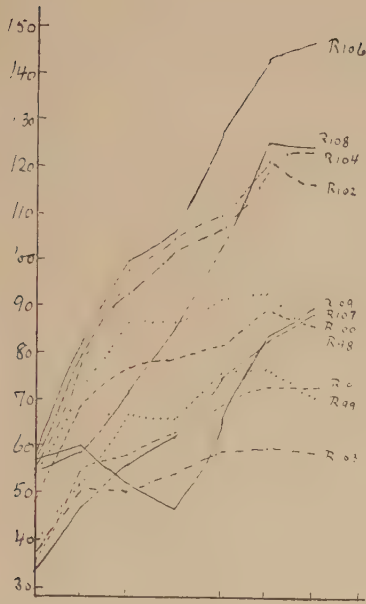


Fig. XIII.

÷ A ÷ P-diet, olive oil with varying additions of cod-liver oil.

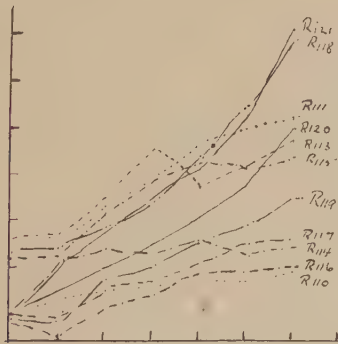


Fig. XIV.

÷ A ÷ Ca-diet, olive oil with varying additions of cod-liver oil.

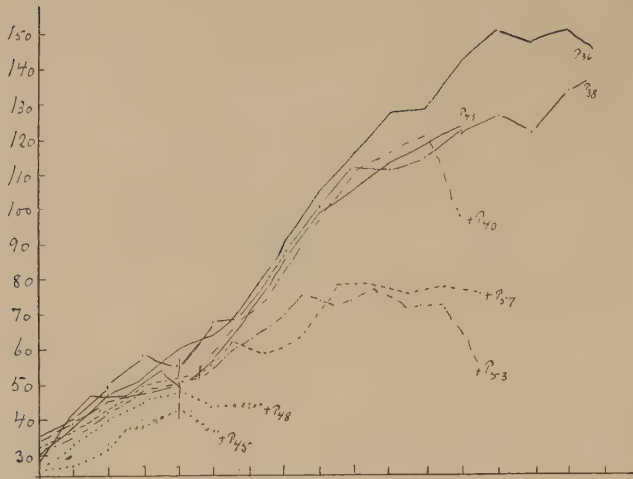


Fig. XVa.

P 45: „A“ 1 mgm of fresh cod-liver oil daily.

P 48: „ 1 „ „ „ „

P 57: „ 1.5 „ „ „ „

P 40: „ 2.0 „ „ „ „

P 53: „ 2.0 „ „ „ „

P 38: „ 5.0 „ „ „ „

P 43: „ 10.0 „ „ „ „

P 36: „ 20.0 „ „ „ „

P 45: $15/5$ xerophthalmia, $19/5$ + addition, $27/5$ autopsy: xerophthalmia, abscess of the root of the tongue, fractures of costae.

P 48: $15/5$ xerophthalmia, $19/5$ + addition, $6/6$ autopsy: xerophthalmia, fractures of the teeth, abscess of the root of the tongue, mastoiditis, prostatitis, vesiculitis semical., cystitis.

P 57: $7/6$ xerophthalmia, $8/6$ + addition, $3/8$ autopsy: panophthalmia, fractures of the teeth, otitis media, abscess of the root of the tongue, cystopyelitis, nephritis.

P 40: $26/4$ xerophthalmia, $1/5$ + addition, $25/5$ the eyes closed, $18/6$ autopsy: xerophthalmia, cystitis.

P 53: $7/6$ xerophthalmia, $8/6$ + addition, $1/8$ autopsy: xerophthalmia, abscess of the root of tongue, cystitis, otitis media.

P 38: $24/4$ xerophthalmia, $1/5$ + addition, $15/5$ the eyes normal, $12/7$ autopsy: 0.

P 43: $15/5$ xerophthalmia, $19/5$ + addition, $25/5$ right eye closed, $9/6$ the eyes normal, $12/7$ autopsy: 0.

P 36: $1/5$ + additton, $12/7$ autopsy: 0.

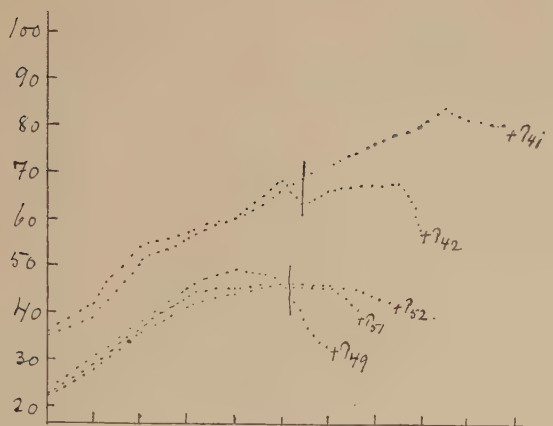


Fig. XVb.

- P 52: „B“ 1 mgm of oxydized cod-liver oil (beg. of experiment 15/4)
- P 42: „ 2 „ „ „ („ 23/3)
- P 51: „ 2 „ „ „ („ 15/4)
- P 41: „ 5 „ „ „ („ 23/3)
- P 49: „ 10 „ „ „ („ 15/4)
- P 52: 15/5 r. eye haemorrhagic. 25/5 panophthalmia. 19/5 + addition. 4/6 autopsy: panophthalmia, fractures of the teeth, abscess of the root of the tongue, mastoiditis, cystitis.
- P 42: 19/4 r. eye haemorrhagic. 1/5 eyes closed. + addition. 19/5: autopsy panophthalmia, fragile teeth, abscess of the root of the tongue, mastoiditis, cystitis.
- P 51: 15/5 eyes haemorrhagic. 19/5 + addition. 23/5 eyes haemorrhagic. 29/5 autopsy: keratomalacia, fragile teeth, abscess of the root of the tongue, cystopyelo-nephritis, osteoporosis.
- P 41: 26/4 xerophthalmia. 1/5 + addition. 20/5 eyes closed. 21/5 autopsy: keratomalacia, fractures of the teeth, abscess of the root of the tongue, fractures of costae, cystitis, prostatitis.
- P 49: 15/5 xerophthalmia. 20/5 eyes closed. 19/5 + addition. 25/5 autopsy: keratomalacia, fractures of the teeth, abscess of the root of the tongue.

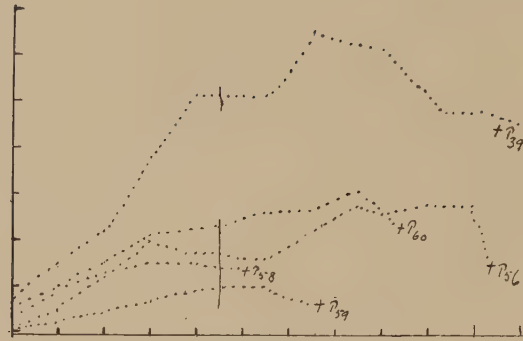


Fig. XVc.

- P 56: „B“ 10 mgm of oxydized cod-liver oil (beg. of experiment $\frac{8}{5}$)
- P 60: „ 10 „ „ „ „ („ $\frac{8}{5}$)
- P 39: „ 20 „ „ „ „ („ $\frac{28}{3}$)
- P 58: „ 50 „ „ „ „ („ $\frac{8}{5}$)
- P 59: „ 50 „ „ „ „ („ $\frac{8}{5}$)
- P 56: $\frac{7}{6}$ xerophthalmia. $\frac{8}{6} +$ addition. $\frac{14}{6}$ eyes closed. $\frac{20}{7}$ autopsy: panophthalmia, fractures of the teeth, abscess of the root of the tongue, cystitis.
- P 60: $\frac{7}{6}$ xerophthalmia. $\frac{8}{6} +$ addition. $\frac{28}{6}$ eyes closed, emaciation. $\frac{9}{7}$ autopsy: panophthalmia, several abscesses.
- P 39: $\frac{1}{6} +$ addition. $\frac{15}{5}$ xerophthalmia. $\frac{2}{6}$ panophthalmia. $\frac{18}{6}$ autopsy: panophthalmia, fractures of the teeth, abscess of the root of the tongue, abscesses of the salivary glands, cystitis, prostatitis, vesiculitis seminal.
- P 58: $\frac{7}{6}$ xerophthalmia. $\frac{8}{6} +$ addition. $\frac{19}{6}$ autopsy: xerophthalmia, abscess of the root of the tongue, osteoporosis.
- P 59: $\frac{7}{6}$ xerophthalmia. $\frac{8}{6} +$ addition. $\frac{19}{6}$ autopsy: xerophthalmia, abscess of the root of the tongue, osteoporosis.

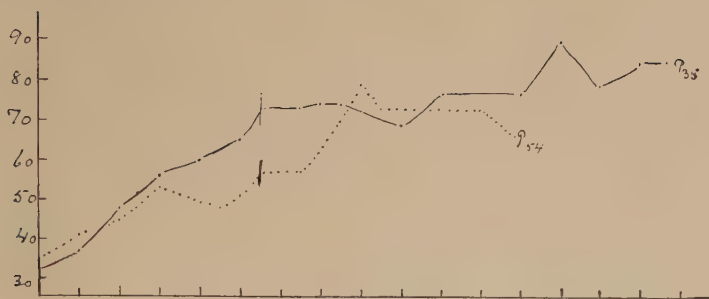


Fig. XVd.

- P 35: ÷ A-diet (from $\frac{1}{5}$ coco-nut oil 0.2 gm daily) in experiment $\frac{23}{3}$.
 P 54: " (" $\frac{8}{8}$ " 0.2 ") " $\frac{8}{5}$.
 P 35: $\frac{2}{6}$ xerophthalmia, $\frac{13}{7}$ autopsy: xerophthalmia, fractures of the teeth, abscess of the root of the tongue, cystopyelitis, nephritis.
 P 54: $\frac{7}{6}$ xerophthalmia, $\frac{19}{7}$ panophthalmia, Autopsy: abscess of the root of the tongue, otitis media, cystopyelo-nephritis, prostatitis, vesiculitis seminal.

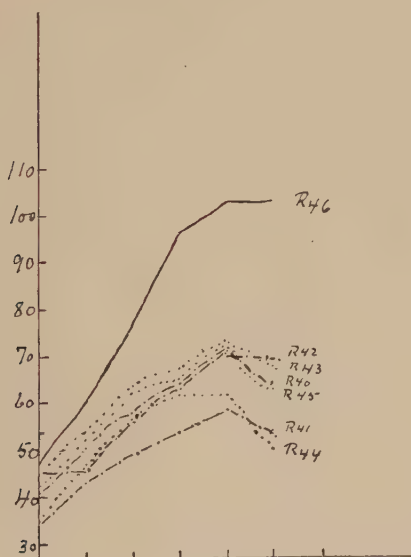


Fig. XVIa.

- ÷ A-diet. R 43, R 44, R 45.
 - . - . - . ÷ A-diet + injection of an unsaponifiable residuum in alcohol.
 ————— N-diet (with 15 p. c. butter fat).

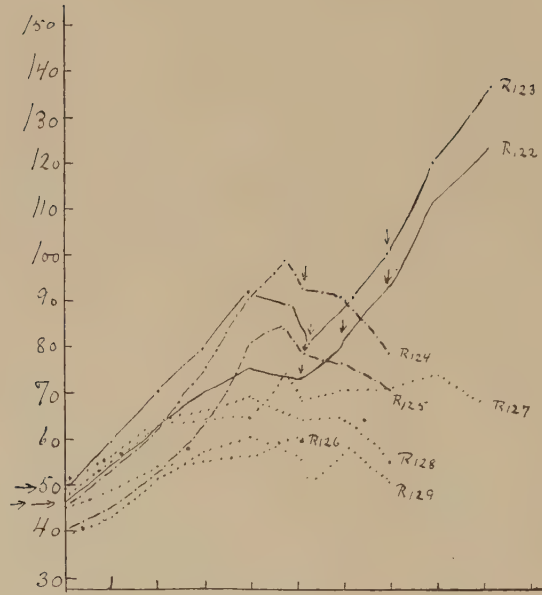


Fig. XVlb

÷ A-diet (+ injections of an unsaponifiable residuum of cod-liver oil).

Injections.

————— R 122	. — . — . — . R 124
. — . — . — . R 123	. — . — . — . R 125

DANISH SUMMARY.

(RÉSUMÉ).

1). Der gives en indgaaende Beskrivelse af den normale Knogleudvikling og Knoglevækst. Paa denne Baggrund beskrives det patologisk-anatomiske Billede af Rachitis. Omtale og Kritik af de eksisterende Teorier om Ætiologien af Rachitis.

2). De to hidtil eneherkende Kostformer (*McCollum's* No. 3143 og *Sherman—Pappenheimers'* No. 84), der er benyttet til Studiet af experimentel Rotterachitis kritiseres som uhensigtsmæssige og uegnede. De er ikke saa syntetisk opbyggede, som det er muligt og de er insufficiante med Hensyn til altfor mange Factorer samtidigt (Æggelhvide, Fedt, A-Vitamin (der er variabelt), B-Vitamin samt med Hensyn til talrige Mineralstoffer). Disse Kostformer vil saaledes ikke kunne give nogen Oplysning om den essentielle ætiologiske Factor.

3). Forsøgsperioden paa dette Regime er altid for kortvarig til at give sikre Oplysninger, specielt om Knoglernes Histologi og Composition.

4). Forsøgsperioden ligger desuden paa et uheldigt Tidspunkt, idet Rotteunger, naar de ca. 30 Dage gamle sættes paa en saadan insufficient Kost, ofte er i Stand til i de følgende 3—5 Uger at vokse lige saa stærkt som Rotteunger paa en adæquat Kost. Man kan saaledes ikke dømme paalideligt om Dyrenes Vægt og Vækst.

5). De anvendte Undersøgelsesmetoder giver Anledning til Fejlslutninger. Den makroskopiske Inspection af Skelettet kan ikke afgøre, om der foreligger normale Knogler eller osteoporotiske Knogler. Det vises, at Osteoporose findes, naar Forsøgsdyrene er standsede i Vækst eller taber i Vægt — og at det

patologisk-anatomiske Billede i saadanne Tilfælde oftest af tidligere Undersøgere er beskrevet som normalt.

6). »The line test« maa betragtes som uanvendelig til at skelne mellem en osteoporotisk og en normal eller helbredt (!) Knogle, idet der ogsaa er paavist Kalkudfældning i Bruskproliferationszonen, naar Dyret i nogen Tid har været holdt paa den rachitogene Kost, uden at der er foretaget noget therapeutisk Indgreb.

7). Røntgenographi kan vel vise, om Bruskproliferationszonen er blevet snævrere og maaske afsløre, om der er optraadt nogen Kalkudfældning; men Røntgenographi kan ikke vise, om Knoglen som Helhed er blevet mineralstofrigere og vandfattigere — altsaa ikke vise, om Knoglen er blevet mere naturlig eller mere osteoporotisk.

8). Vurderingen af Blodets Indhold af Kalk og Fosfor kan endnu ikke benyttes som Diagnosticon for Rachitis eller Helbredelse heraf; dertil er dette Indhold altfor paavirkeligt af ucontrollable Indflydelser: Forsøgets Varighed, Sult, Lyspaavirkning, Ændringer i Kostens Saltindhold, Tilførsel af Levertran et cetera.

9). I de fleste tidligere Forsøg er undladt de to vigtigste og sikreste Undersøgelsesmetoder: histologisk Knogleundersøgelse og den chemiske Knogleundersøgelse. Den histologiske Knogleundersøgelse kan vise, om det drejer sig om en normal Knogle med normal Vækst eller om en rachitisk Knogle med nogen, men retarderet og ufuldstændigt differentieret Vækst eller endelig om en osteoporotisk Knogle med Rarefaction, lacunær Knogleresorption og standset Vækst i Brusken.

Den chemiske Knogleundersøgelse vil give Oplysning om Knoglens Vandindhold og Askeindhold. Denne Undersøgelsesmetode er lige saa væsentlig som den histologiske, idet kun den kan give Oplysning om, hvorvidt en forud rachitisk Knogle ved en eller anden therapeutisk Forholdsregel er blevet vandfattigere (i. e. helbredt), eller hvorvidt Vandindholdet er steget og Mineralindholdet dalet (i. e. Knoglen blevet mere osteoporotisk). Det gælder her Askeindholdet, beregnet efter frisk Knogle og ikke efter tør, fedtextraheret Knogle. En meget osteoporotisk

Knogle kan være saa vandholdig og indeholde saa lidt organisk Substans, at Askeindholdet, beregnet procentisk efter tør, extraheret Knogle, synes højt, medens det, beregnet i Forhold til den friske Knogle er meget lavt.

10). Det vises saaledes, at man sandsynligvis har draget fejle Slutninger i talrige og de tidligere Forsøg paa disse rachitogene Regimer, og at man — paa Grund af disse Regimers meget hæmmende Art — i hvert Fald ikke har kunnet afgøre de stillede Spørgsmaal.

11). De her meddelte Forsøg er udførte paa en adæquat og syntetisk Kost med adæquat Saltindhold (Pag. 60), og man har saaledes kunnet ændre een eller to kendte Factorer ad Gangen, og derved kunnet bedømme Virkningen heraf.

12). Det vises, at Rotteunger paa en A-vitamin-fri, men iøvrigt adæquat Kost, faar udviklet de kendte Symptomer paa A-Avitaminose, og det understreges, at de fremkaldte Knogleforandringer er: *Osteoporose*, selvom der hos nogle af Dyrene findes endnu bevarede, lidet differentierede Bruskceller i uregelmæssige Søjler i den Zone, der svarer til den provisoriske Forkalkningszone.

13). Rotteunger paa en A-vitaminfri og kalkfattig Kost (0.04 % Ca) viser — som det maatte ventes, naar Kosten er blevet endnu mere insufficient — næsten ingen Vækst. Knoglerne er i høj Grad osteoporotiske, og dette Billede tilsløres blot for en flygtig Iagttagelse af en højere Opbygning af endnu bevarede, udifferentierede Bruskceller dels i Epifyselinien, dels i Marvrummet.

14). Noget lignende Billede, men i mindre voldsom Grad af Osteoporose kan træffes hos Rotteunger paa A-fri og fosforfattig Kost (0.2 % P).

15). Ved talrige Undersøgelser over forskellige Smørprøvers Indhold af A-Vitamin — idet Smørret er givet som daglige Tillæg af 0.025—0.5 gr. til vor A-frie, men iøvrigt adæquate Kost — finder man, at der kræves en ganske bestemt Dosis — forskellig for de forskellige Smørsorter — for at forhindre Indtræden af A-Avitaminose. a) Dyr, der faar mindre end et saadant Tillæg eller slet intet Tillæg, viser udtalt *Osteoporose*. b) Dyr,

der har faaet noget større Tillæg, saaledes at Vægten enten bevares eller er noget stigende, viser *udtalt florid Rachitis* — men undgaar at faa de øvrige af A-Avitaminosens iøjnefaldende Symptomer. c) Dyr, der faar saa store Tillæg, at Vægtstigningen er normal, viser ogsaa *normalt Knoglebillede og normal Composition af Knoglerne*.

16). *Det vises saaledes, at florid Rachitis kun kan fremkal-des ved Tilførsel af visse Mængder A-Vitamin!!*

De forskellige Tilstande i Knoglesystemet: Osteoporose, Rachitis, normal Knoglebygning og -vækst er saaledes alene en Følge af respective: ingen eller subminimal Tilførsel af A-Vitamin — af nogen, men suboptimal Tilførsel — og endelig af optimal og sufficient Tilførsel — af A-Vitamin. *Det er et Dose-ringsspørgsmaal.*

17) *Ganske lignende Forhold vises for Levertrans Vedkom-mende.* Ogsaa her vil saadanne Mængder Levertran, der er tilstrækkelige til at sikre en vis Vækst — men for smaa til at give normal Vækst — bevirke, at der udvikles Rachitis.

18). Har man fremkaldt Osteoporose paa en A-fri Kost og derefter erstatter det A-frie Fedtstof med adæquat A-holdigt Fedt, vil Knoglevæksten straks indtræde, og den fortsættes paa normal Maade.

19). I Forsøg over Indvirkningen af Kostformer, der indeholder forskellige Mængder A-Vitamin (Levertran), men insuf-ficiente Saltmængder (dels med Hensyn til Ca, dels P), finder man ikke alene ved den histologiske Undersøgelse, men ogsaa ved chemiske Analyser, at det rachitiske Billede er udtalt, og at den chemiske Composition er betydeligt mere forringet, end om Kosten havde indeholdt adæquate Saltblandinger. Tillige vi-ses det, at *der kræves et større A-Vitaminindhold i Kosten for at beskytte Organismen mod denne Saltmangels skadelige Ind-flydelse. Forøget Tilførsel af A-Vitamin er kun en Fordel for Organismen, saafremt den indgivne Saltblanding er adæquat.*

20). Paa Basis af egne Undersøgelser og paa Basis af Kritik-ken af andres Undersøgelser hævdes det, at der kun kan findes Holdepunkter for Antagelsen af eet fedtopløseligt, vækstfrem-kaldende Vitamin.

21). Dette vises yderligere ved Forsøg over iltet Levertran. Ved Iltningen er det vækstfremkaldende Vitamin destrueret — men samtidigt har Levertranen ogsaa ganske mistet Evnen til at formindske Knoglernes Vandindhold og til at forøge deres Askeindhold. Den iltede Levertran viste stærk »Photoaktivitet« paa en fotografisk Plade.

22). Da Rachitis saaledes kun findes hos Dyr, der vokser, og da Rachitis netop maa betragtes som Følgen af den disproportionerede Vækst, der indtræder, naar Organismen tilføres visse Mængder A-Vitamin, der viser sig utilstrækkelige for den normale Væksthastighed og Celle-differentiation, vil man se: *at der kun kan tales om en virkelig Helbredelse af Rachitis, naar det lykkes at forøge Vækstens Differentiationsprocesser.* Det forudsættes her, at de øvrige fornødne Componenter til Organismens Opbygning — specielt Mineralstofferne — er til Stede i Kosten i adæquate Mængder.

23). Da rachitiske Knogler udvikles til osteoporotiske Knogler, naar Dyret er standset i Vækst og taber i Vægt, vil man forstaa, at et Stof eller en therapeutisk Forholdsregel kun kan virke »antirachitisk«, *helbredende* i. e. forbedrende Knoglernes histologiske Bygning og kemiske Composition — naar det samtidig lykkes at fremkalde Vækstprocesser.

24). Der vises, at alle Symptomer, der udvikles ved A-Avitaminose: Xerophthalmi, Kirtelatrofi, Osteoporose, Hypoplasi af Knoglemarven, Anæmi, de talrigt indtrædende suppurative Betændelser i Legemets Hulheder og Kirtelorganer er en simpel Følge af den universelle Vækststandsning. Herved optræder en universel, regressiv Metaplasi af Epithelet i Udførselsgange og i Kirtelacini, hvorved Bacteriernes Indtrængen lettes, og Betændelser ofte indtræder. *Alle disse Symptomer svinder prompte for A-Vitamintilførsel.*

25). Endeligt diskuteres Betydningen af de opnaaede Resultater for Therapien og Profylaxen af A-Avitaminosen og specielt af Rachitis hos Børn.
